

EFFECT OF LIVER CIRRHOSIS AND  
TRANSPLANTATION ON FUEL METABOLISM AND  
MACRONUTRIENT PREFERENCE

Rosemary Ann Richardson, MSc, SRD.

A thesis submitted to the University of Edinburgh for the  
degree of Doctor of Philosophy

Department of Clinical and Surgical Sciences (Surgery),  
Royal Infirmary,  
Edinburgh EH3 9YW.

October 1999



To my dear parents

Marjory and Douglas



## LIST OF CONTENTS

	Page
List of Contents	3
List of Tables	5
List of Figures	7
List of Abbreviations	11
Acknowledgements	13
Signed Declaration	14
Declaration of Work Published and Presented	15
Statement of Collaboration	18
Abstract	19
Chapter 1    Review of the Literature	21
Aims of the Thesis	66
Chapter 2    Methods	68
Subjects	68
Indirect calorimetry studies	69
Anthropometry	74
Multi-frequency bioelectrical impedance analysis	76
Biochemical measurements	77
Test meal	81
Nutrient intake	82
Macronutrient preference	82
Statistics	84
Chapter 3    The Clinical and Nutritional Status in Liver Cirrhosis and Liver Transplant Recipients	86
Chapter 4    Diet Induced Thermogenesis in Liver Cirrhosis and Liver Transplant Recipients	115

	<b>Page</b>
Chapter 5    Fasting Substrate Utilisation in Liver Cirrhosis and Liver Transplant Recipients	136
Chapter 6    Substrate Utilisation in Response to Feeding in Liver Cirrhosis and Liver Transplant Recipients	148
Chapter 7    Insulin and Glucose Response to Feeding in Liver Cirrhosis and Liver Transplant Recipients	166
Chapter 8    Energy and Macronutrient Intake in Liver Cirrhosis and Liver Transplant Recipients	190
Chapter 9    Macronutrient Preference in Liver Cirrhosis and Liver Transplant Recipients	213
Chapter 10   The Influence of Drug Regimens on Body Mass Following Liver Transplantation	236
Chapter 11   Discussion and Conclusions	247
References	257
Appendix	280

## LIST OF TABLES

Table		Page
1.1	Child's Pugh classification of cirrhosis	29
2.1	Macronutrient values of ice creams	83
3.1	Nutritional and clinical status of cirrhotic patients and controls	88
3.2	Metabolic status of cirrhotic patients and controls	89
3.3	Comparisons between MFBIA results for male cirrhotic patients and controls, and female cirrhotic patients and controls	91
3.4	Nutritional, metabolic and clinical status of cirrhotic patients classified by Child's Pugh score	95
3.5	Nutritional, metabolic and clinical status of cirrhotic patients classified by primary aetiology	97
3.6	Characteristics of patients following liver transplantation	99
3.7	Changes in body mass and arm anthropometry before and following liver transplantation	100
3.8	Changes in body composition before and following liver transplantation	101
4.1	Characteristics of cirrhotic patients and control subjects	118
4.2	Energy expenditure in response to feeding pre- and post-liver transplantation	127
5.1	Fasted substrate oxidation rates per unit body mass of control subjects and cirrhotic patients	142
5.2	Fasting NPRQ and substrate oxidation rates in control subjects and patients following liver transplantation	143

Table		Page
6.1	Non-protein respiratory quotient, glucose and lipid oxidation rates in response to feeding following liver transplantation	159
8.1	Energy and macronutrient intake in cirrhotic patients before and following liver transplantation	200
9.1	Hedonic ratings for test stimuli in control subjects and patients following liver transplantation	220
10.1	Changes in body composition, energy and fat intake before and following liver transplantation	239
10.2	Immunosuppressive therapy following liver transplantation	240
10.3	Stepwise multivariate regression analysis with indices of fat mass (nine months following liver transplantation) as the dependent variable	243

## LIST OF FIGURES

Figure		Page
1.1	Liver-brain axis	26
1.2	Schematic representation of mode of action of BIA where increasing frequency allows current to pass through cell membrane	41
1.3	Post-absorptive flow of substrates between the liver, CNS, adipose tissue, muscle and red blood cells	47
1.4	Physiological influences on nutritional intake	61
2.1	Schematic outline of study protocol	71
3.1	Measured energy expenditure versus primary pathology and disease severity in cirrhotic patients	92
3.2	The relationship between measured energy expenditure and body cell mass	94
3.3	Comparison of measured and predicted energy expenditure	103
4.1	Thermogenic response to feeding in control subjects and cirrhotic patients	119
4.2	Thermogenic response to feeding in cirrhotic patients stratified by disease severity	121
4.3	Thermogenic response to feeding in control subjects and cirrhotic subjects with biliary or hepatocellular cirrhosis	122
4.4	Oxygen consumption in response to feeding in control subjects, biliary and hepatocellular cirrhotic patients	123
4.5	Respiratory quotient in response to feeding in control subjects, biliary and hepatocellular cirrhotic patients	124

Figure		Page
4.6	Thermogenic response to feeding in patients before and following liver transplantation	126
5.1	Substrate oxidation in control subjects and cirrhotic patients	138
5.2	Substrate oxidation in cirrhotic patients stratified by disease severity	140
6.1	Glucose oxidation in controls and cirrhotic patients	150
6.2	Lipid oxidation in controls and cirrhotic patients	151
6.3	Glucose oxidation in patients stratified by disease severity	153
6.4	Lipid oxidation in patients stratified by disease severity	154
6.5	Glucose oxidation in patients with biliary and hepatocellular cirrhosis	155
6.6	Lipid oxidation in patients with biliary and hepatocellular cirrhosis	157
6.7	Change in lipid and glucose oxidation rates in patients with biliary and hepatocellular cirrhosis	158
7.1	Glucose response to feeding in controls and cirrhotic patients	169
7.2	Insulin response to feeding in controls and cirrhotic patients	170
7.3	Glucose response to feeding in cirrhotic patients stratified by disease severity	171
7.4	Insulin response to feeding in cirrhotic patients stratified by disease severity	172
7.5	Glucose response to feeding in cirrhotic patients without and with portasystemic shunting	174

Figure		Page
7.6	Insulin response to feeding in cirrhotic patients without and with portasystemic shunting	175
7.7	Glucose response to feeding in controls and patients with biliary and hepatocellular cirrhosis	176
7.8	Insulin response to feeding in controls and patients with biliary and hepatocellular cirrhosis	177
7.9	Glucose response to feeding before and following liver transplantation	180
7.10	Insulin response to a mixed meal before and following liver transplantation	182
8.1	Energy and macronutrient intake in control subjects and cirrhotic patients	194
8.2	Energy and macronutrient intake in cirrhotic patients stratified by Child's Pugh score	195
8.3	Energy and macronutrient intake in control subjects and patients stratified by aetiology	196
8.4	Energy balance in control subjects and patients stratified by aetiology	198
8.5	Nitrogen balance	199
8.6	Energy and macronutrient intake before and following liver transplantation	201
8.7	Contribution of macronutrient to total energy intake before and following liver transplantation	203
9.1	Preference for high fat, moderate carbohydrate in control subjects, biliary and hepatocellular cirrhotic patients	216

Figure		Page
9.2	Macronutrient preference in cirrhotic patients	218
9.3	Associations between substrate oxidation and test stimuli	219
9.4	Macronutrient preference for each test stimuli following liver transplantation	222
9.5	Macronutrient preference three, six and nine months following liver transplantation	223
9.6	Macronutrient preference in the biliary cirrhotic group following liver transplantation	225
9.7	Macronutrient preference in the hepatocellular cirrhotic group following liver transplantation	226
9.8	Macronutrient preference in biliary and hepatocellular patients before and at nine months following liver transplantation	227
10.1	Resting energy expenditure versus fat mass nine months following liver transplantation	242



## LIST OF ABBREVIATIONS

ALD	=	alcoholic liver disease
AMC	=	arm muscle circumference
ATP	=	adenosine tri-phosphate
ANOVA	=	analysis of variance
AU	=	arbitrary units
AUC	=	area under curve
BC	=	biliary cirrhosis
BCM	=	body cell mass
BIA	=	bioelectrical impedance analysis
BMI	=	body mass index
C	=	carbon
CCK	=	cholecystokinin
CHO	=	carbohydrate
CO <sub>2</sub>	=	carbon dioxide
CoV	=	co-efficient of variation
CsA	=	cyclosporin A
DM	=	diabetes mellitus
ECW	=	extracellular water
FFM	=	free fat mass
g	=	gram
H <sub>2</sub> O	=	water
HC	=	hepatocellular cirrhosis
HF HCHO	=	high fat high carbohydrate
HF modCHO	=	high fat moderate carbohydrate
K	=	potassium
kg	=	kilogram
L	=	litre
LBM	=	lean body mass
LF modCHO	=	low fat moderate carbohydrate
LF HCHO	=	low fat high carbohydrate
LTx	=	liver transplantation
ICW	=	intracellular water
IQR	=	interquartile range
kJ	=	kilojoule

MFBLA	=	multifrequency bioelectrical impedance analysis
min	=	minute
MJ	=	megajoule
N	=	nitrogen
NPRQ	=	non-protein respiratory quotient
O <sub>2</sub>	=	oxygen
OLT	=	orthotopic liver transplantation
PBC	=	primary biliary cirrhosis
REE	=	resting energy expenditure
RQ	=	respiratory quotient
SEE	=	standard error of estimate
SEM	=	standard error of the mean
SLTU	=	Scottish Liver Transplant Unit
SNS	=	sympathetic nervous system
TBK	=	total body potassium
TBN	=	total body nitrogen
TBW	=	total body water
TSF	=	triceps skinfold thickness
VCO <sub>2</sub>	=	volume carbon dioxide
VO <sub>2</sub>	=	volume oxygen

## ACKNOWLEDGEMENTS

I wish to express my sincere gratitude to the following:

Professor O. James Garden, Professor of Hepatobiliary Surgery, Department of Clinical and Surgical Sciences (Surgery), University of Edinburgh for acting as my supervisor, for his wise counsel and for always having an open door.

Dr. Isobel Davidson, Lecturer in Physiology, Queen Margaret University College, for her supervision, endless patience, reassuring advice and valued friendship.

Dr. Peter Rae, Consultant Biochemist, Western General Hospital for advice on blood analysis and Dr. Alastair MacGilchrist, Consultant Hepatologist, Royal Infirmary of Edinburgh for his advice. Dr. Jim Ross, Non-Clinical Senior Lecturer, University of Edinburgh for allowing me to use the facilities of the Lister Laboratories.

The Department of Dietetics and Nutrition, Queen Margaret University College and to my colleagues whose support allowed me to complete this thesis. Mr. Donald Sutherland, Statistician, Queen Margaret University College for his advice.

Nutricia Ltd. and in particular my friend and colleague, Mrs Christine Russell.

Mrs Susan Keggie for her artistic input on the figures and patience.

Miss Anne McKellar for her secretarial wizardry, powers of endurance and her friendship.

## SIGNED DECLARATION

This thesis has been composed and written entirely by myself  
and has not been submitted previously for any other degree.

Date: 18<sup>th</sup> May 2000 .....

## DECLARATION OF WORK PUBLISHED AND PRESENTED

Listed below are the publications, published abstracts and presentations to societies to date. Some of the work contained in this thesis is currently under consideration by scientific journals.

### Publications

1. Davidson HIM, Pattison RM, Richardson RA. 1998 Undernutrition and its influence on taste. *Proceedings of the Nutrition Society* 57; 633-638.
2. Richardson RA, Davidson HIM, Hinds A, Cowan S, Rae P, Garden OJ. 1999 The influence of the metabolic sequelae of liver cirrhosis on nutritional intake. *American Journal of Clinical Nutrition* 69; 331-337.
3. Davidson HIM, Richardson RA, Sutherland D, Garden OJ. 1999 Macronutrient preference, dietary intake and substrate oxidation among stable cirrhotic patients. *Hepatology* 29; 1380-1386.
4. Walsh TS, Wigmore SJ, Hopton P, Richardson RA, Lee, A. 2000 Energy expenditure in paracetamol induced fulminant hepatic failure. *Critical Care Medicine* 28; 649-654.

### Papers In Preparation

1. Richardson RA, Davidson HIM, Garden OJ. 1999 Obesity after liver transplantation: a denervation phenomenon. *Hepatology*.
2. Davidson HIM, Richardson RA, Garden OJ. 1999 Changes in plasma leptin concentrations following human orthotopic liver transplantation. *Transplantation*.
3. Richardson RA, HIM Davidson, Garden OJ. 1999 The metabolic control of eating - from basic science to clinical appliance. *Nutrition*.

## Presentations and Published Abstracts

The following papers have been presented and published in abstract form where indicated.

1. Richardson RA, Davidson HIM, Cowan S, MacGilchrist A, Garden OJ. Multi-frequency bioelectrical impedance analysis in estimating body composition in patients with chronic liver disease. Danish Association of Parenteral and Enteral Nutrition, Aarhus, March 1997. Proceedings of the Danish Association of Parenteral and Enteral Nutrition, 1997.
2. Richardson, RA, Davidson HIM, Hinds A, Bate R, MacGilchrist A, Garden OJ. Body composition changes and increased energy efficiency influence weight gain following liver transplantation. United European Gastroenterology Week, Birmingham, October 1997. Gut 1997, 41: 3(Suppl.), A428.
3. Richardson RA, Davidson HIM, Hinds A, MacGilchrist A, Garden OJ. Energy economy following liver transplantation. British Association of Parenteral and Enteral Nutrition, Blackpool, December 1997. Proceedings of the Nutrition Society 1998 57 : 3; 88A.
4. Davidson HIM, Richardson RA, Hinds A, MacGilchrist A, Garden OJ. Changes in body composition following liver transplantation. British Association of Parenteral and Enteral Nutrition, Blackpool, December 1997. Proceedings of the Nutrition Society 1998 57: 3;101A.
5. Richardson RA, Davidson HIM, Garden OJ. The role of the liver in the metabolic control of eating. Irish Dietetic Association, Dublin, April 1998.
6. Richardson RA. Metabolic research in liver disease. University Department of Surgery, Royal Infirmary of Edinburgh, June 1998.
7. Richardson RA. Influence of aberrant metabolism on nutritional intake and status in liver disease. Department of Gastroenterology, Western General Hospital, Edinburgh, July 1998.
8. Richardson RA, Davidson HIM, Garden OJ. The impact of biliary and hepatocellular cirrhosis on nutritional intake and macronutrient preference. European Society of Parenteral and Enteral Nutrition, Nice, France, September 1998. Clinical Nutrition 1998, 17 (suppl 1) 18.
9. Richardson RA, Davidson HIM, Garden OJ. Metabolic response to feeding in biliary and hepatocellular cirrhosis. European Society of Parenteral and Enteral Nutrition, Nice, France, September 1998. Clinical Nutrition 1998, 17 (suppl 1) 65.

10. Davidson HIM, Richardson RA, Garden OJ. Leptin increases sequentially after liver transplantation. European Society of Parenteral and Enteral Nutrition, Nice, France, September 1998. *Clinical Nutrition* 1998, 17 (suppl 1) 43.
11. Walsh TS, Wigmore SJ, Hopton P, Richardson RA and Lee A. Energy expenditure in paracetamol induced fulminant hepatic failure. *Intensive Care Medicine* 1998, 24 (S1).
12. Richardson RA. Dietary management of liver disease - a new perspective. British Dietetic Association (South West of England Branch). Bristol February 1999.
13. Richardson RA. Metabolic control of ingestive behaviour. British Association of Parenteral and Enteral Nutrition Education Course. Grasmere February 1999.
14. Richardson RA, Davidson HIM, Garden OJ. Energy dynamics in liver transplanted patients. European Society of Parenteral and Enteral Nutrition, Stockholm, Sweden, September 1999. *Clinical Nutrition* 1999, 18 (suppl 1) 113.
15. Davidson HIM, Richardson RA, Garden OJ. Qualitative and quantitative changes in energy intake following human denervation. *Proceedings of the Nutrition Society* 2000 (in press).

## STATEMENT OF COLLABORATION

All study designs were constructed by myself with advice from Dr. H.I.M. Davidson and Professor O.J. Garden. The study design involving the test meal was constructed with advice from Professor I. MacDonald, Department of Physiology, Queen's Medical Centre, Nottingham.

All calorimetry measurements were performed by myself and Dr. A. Hinds.

Cannulation of the ante-cubital vein was performed by a member of the medical staff in the Transplant Unit. All blood sampling and initial processing of these samples was performed by myself. Insulin and glucose measurements were performed in the Department of Clinical Chemistry, Western General Hospital, Edinburgh. Measurement of C-reactive protein was undertaken by myself with advice from Dr. A. Hinds.

The urine samples were analysed by myself with the assistance of Mr. Colin Nicholson (technical staff) in the Department of Dietetics and Nutrition, Queen Margaret College, Edinburgh.

All anthropometric measurements and analysis of dietary diaries were performed by myself. All test meals and food preference samples were prepared by myself.

All figures and graphs have been produced by myself.

All the references cited in the text have been read by myself.

Statistical advice was provided by Professor Gordon Murray, Department of Medical Statistics, University of Edinburgh and Mr. Donald Sutherland, Queen Margaret University College, Edinburgh.



## ABSTRACT

The liver is an important metabolic sensor relaying humoral and neural information via the brain stem to the hypothalamus. In response to fasting or feeding it is the appropriate orchestration of these events that controls ingestive behaviour and maintains energy homeostasis. In chronic liver disease, alterations in oxidative metabolism and impairment of the liver's metabolic role may effect changes in ingestive behaviour and nutritional status. Liver transplant recipients have lost all hepatic innervation and the absence of humoral and neural responses relayed via the hepatic afferents could effect energy balance and contribute to post-liver transplant obesity. This study examined longitudinal changes in body composition, dietary intake, macronutrient preference and energy metabolism in the fasted and fed state.

Sixty-seven patients with chronic liver disease and a group of 18 healthy volunteers were recruited. A sub-group of 23 patients who underwent liver transplantation were reviewed every three months on three occasions. Nutritional status was determined using body habitus measurements and multi-frequency impedance analysis. Indirect calorimetry was used to determine energy and substrate metabolism in the fasted and fed state. Basal and post-prandial insulin and glucose concentrations were measured. Diet diaries were used to estimate energy and macronutrient intake and macronutrient preference was identified using food varying in fat and carbohydrate content.

Patients with cirrhosis of hepatocellular origin (alcoholic liver cirrhosis, cryptogenic cirrhosis) had the greatest disturbances in substrate metabolism, depressed dietary intakes and poorest nutritional status when compared with patients with biliary cirrhosis and control subjects. These findings suggest that aberrant metabolism may contribute to anorexia and impact on nutritional status in patients with hepatocellular cirrhosis. No differential effect was observed when patients were stratified for severity of disease.

Following liver transplantation, patients weight exceeded pre-illness values by 7% and this increase in body weight was accounted for by fat mass but not lean tissue. A decrease in resting energy expenditure was observed and a 5% increase in the dietary intake of fat derived energy. No association between immunosuppressive medication and body weight was seen. Multiple regression analysis revealed that the strongest predictor of weight gain was resting energy expenditure. These findings suggest that the liver transplant procedure *per se* is implicated in the energy economy and fat hyperphagia observed following liver transplantation which may be a result of denervation and the loss of afferent input and efferent outflow.

## CHAPTER 1

### REVIEW OF THE LITERATURE

#### Effects of Chronic Liver Disease and Transplantation on Fuel Metabolism and Macronutrient Preference

#### INTRODUCTION

Throughout history the liver has not only been perceived as an organ essential to life but also one which possesses mystical powers. In ancient Rome a priest would use an animal liver in the same fashion as a modern day crystal ball to predict the outcome of a debate in the Senate or a major battle. Even today the liver is associated with our emotions and terms such as "lily livered" or "yellow" are used to describe cowardice and "gall" arrogance. The liver's predictive abilities and its relationship with emotional characteristics are no longer considered important. However, if the pathophysiological sequelae of liver disease are considered, it is apparent that these will influence emotional state; for example milder forms of hepatic encephalopathy can cause changes in mood and severe cases result in profound confusion and coma.

The liver is the body's largest organ weighing between 1.2 - 1.8 kg. The liver is pivotal in nutrient and drug metabolism, synthesis and release of plasma protein as well as playing a key role in the innate immune system by harbouring Kupfer cells. The liver is fed nutrient rich blood from the portal vein and designates nutrients to be oxidised, stored or redistributed into the peripheral circulation. Normal liver function depends on the appropriate utilisation of nutrients to provide energy for

its own macromolecular synthesis. As an organ involved in the synthesis and degradation of nutrients its function must be co ordinated and regulated in response to metabolic changes. This complex metabolic interplay may be compromised in liver disease.

In chronic liver disease or cirrhosis, there is progressive and eventually irreversible liver damage and patients may present with jaundice, oedema, ascites, central nervous system dysfunction as well as undernutrition. Causes of cirrhosis include alcoholic liver disease (ALD), primary biliary cirrhosis (PBC), primary sclerosing cholangitis, cryptogenic cirrhosis, chronic hepatitis and Wilson's disease.

Alcoholic liver cirrhosis and PBC are the commonest referrals for elective liver transplantation in Scotland (Scottish Liver Transplant Unit Annual Report, 1998) and remains the most common causes of cirrhosis in Europe. In a large Italian study on 1,492 cirrhotics, alcohol-related disease accounted for over a third (37%) of the patient population (Merli et al, 1996). Similarly an earlier study from the United Kingdom on 463 cirrhotics (Saunders et al, 1981) found that more than half these patients presented with ALD (245 out of 463).

The origins of ALD are a consequence of long term alcohol abuse resulting in toxic damage to the hepatocyte. Primary biliary cirrhosis is a progressive disease generally affecting women between the fourth and seventh decade of life where continued portal and peri-portal inflammation and cholestasis leads to the development of cirrhosis, portal hypertension and liver failure (Kaplan, 1987).

In metabolic studies of chronic liver disease, investigators have grouped together cirrhotic patients with varying pathologies (Schneeweiss et al, 1990, Muller et al 1992, Verboeket-van de Venne et al,

1995). Whilst this may be considered important in improving the statistical power of studies, it could mask adaptive metabolic differences between these different pathophysiological populations. For example, ALD is characterised by intrinsic hepatocellular damage whereas patients with PBC present with features of cholestasis. It is only when liver disease becomes severe that the end-stage features of hepatocellular and cholestatic disease may be similar.

The progression of chronic liver disease is variable but where longterm prognosis is poor, orthotopic liver transplantation (OLT) is an important and worthwhile treatment modality. Belle and colleagues (1997) in a multi-centred study in 346 OLT patients found that quality of life significantly improved one year after OLT when compared with pretransplant values. Prior to undertaking OLT it is essential that potential recipients are referred early and undergo stringent physical and psychological assessment. Such rigorous assessment serves not only to indentify the suitability of patients for transplantation, it also permits identification of patient's 'needs' and allows these to be met thereby optimising the patient's pre-operative physical and mental state.

Whilst many of the symptoms of cirrhosis can be adequately controlled by drugs, one distressing problem affecting many patients is a continued deterioration in nutritional status. Poor nutritional state in liver disease is not uncommon (Crawford et al 1993; Thuluvath & Triger 1994; Mendenhall et al 1995) but improving the quantity and quality of dietary intake of patients with cirrhosis is difficult.

## Undernutrition in Liver Cirrhosis

The manifestation of under-nutrition in cirrhosis is generally insidious and rarely requires immediate medical management. Consequently patients diminishing body mass is often regarded by the clinician as a natural component of chronic liver disease and frequently goes unnoticed and untreated. Moreover, the presence of undernutrition may be masked by ascites and/or peripheral oedema. Factors implicated in the development of undernutrition in chronic liver disease include:

1. malabsorption - which may occur as a result of reduced bile secretion or as a direct effect of a high alcohol intake.
2. reduced dietary intake - symptoms of chronic liver disease such as jaundice, ascites and encephalopathy may detrimentally influence nutritional intake.
3. increased energy requirements - several investigators have suggested that chronic liver disease is a hypermetabolic disease (Shanbhogue et al 1987, Green et al 1991, Muller et al 1994). Although an area of controversy, a continued negative energy balance could significantly contribute to undernutrition in chronic liver disease. For example, Green et al (1991) in a small study of seven patients with PBC suggested that when compared with a group of age and sex matched controls these patients were hypermetabolic. Interestingly, extrapolation of the energy data from the study of Green and colleagues suggests that the energy deficit experienced by these patients would result in a loss of body mass of 35 - 75g/day (0.5 kg in one week). Obviously this calculation is an oversimplification of energy balance dynamics but it does contextualise the cumulative effect of a negative energy balance on body mass. In the study of Green and colleagues (1991) patients were energy

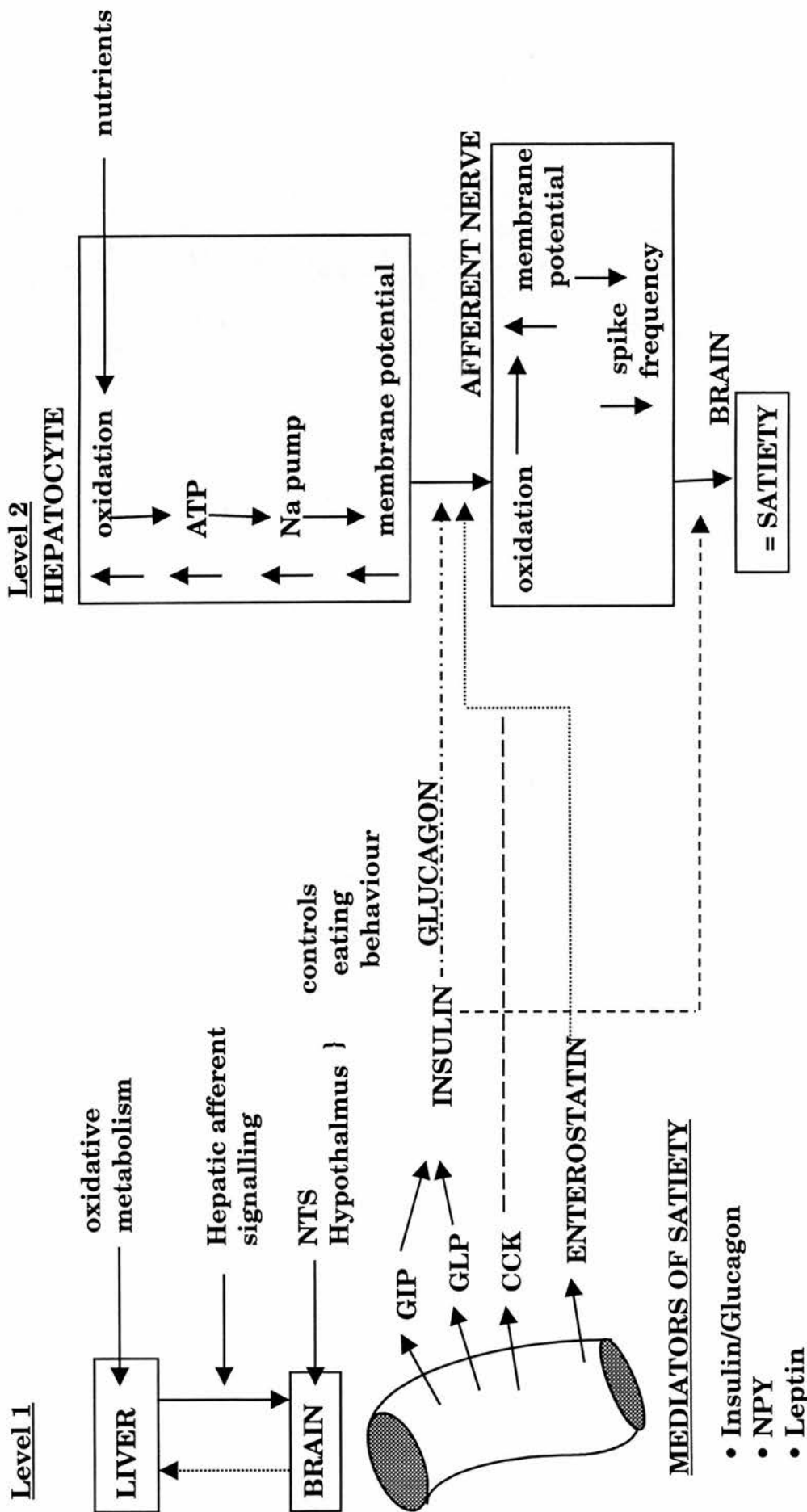
(fat stores) but not protein (muscle stores) depleted suggesting these patients were exhibiting features of simple starvation. It may be that the hypermetabolism observed by these investigators may simply reflect a higher ratio of lean to fat mass.

4. altered metabolism - commonly reported in chronic liver disease and may be important in the development of undernutrition. For example, as chronic liver disease progresses, the synthetic capacity of the liver to store glycogen diminishes, there is a reduction in hepatic glucose production, enhanced gluconeogenesis and increased lipolysis (Ohyanagi et al, 1995). In cirrhosis the metabolic fuels used to meet fasting energy requirements switch from predominantly glucose to increased mobilisation and oxidation of both endogenous fat and protein stores. A prolonged and continued reliance on endogenous fat and protein stores will, if accompanied by a negative energy balance, lead to depletion of body mass (Owen 1981; Green 1991).

5. role of the liver as a metabolic sensor - there is unequivocal evidence to support the role of the liver in the control of food intake (Novin et al, 1985; Friedman et al, 1997) where, as a result of hepatic substrate oxidation, changes in the hepatocytes membrane potential occurs causing a reduction in afferent spike frequency and results in transmission of a satiety signal to the brain (Niiijima, 1982) (Figure 1.1). It could be postulated that in chronic liver disease, where there are alterations in circulating metabolites this could effect changes in humoral and neural metabolic signalling, affect satiety mechanisms, influence nutritional intake and bring about changes in body composition.

FIGURE 1.1

LIVER-BRAIN AXIS



NTS = nucleus tract solarius, GIP = gastric inhibitory peptide, GLP = glucagon-like-peptide  
 CCK = cholecystokinin, NPY = neuropeptide Y, Na = sodium, ATP = adenosine-tri-phosphate



Convincing evidence to support the role of the liver as a metabolic sensor comes from important small animal work by Nijima (1982), where electrophysiological studies showed that when glucose was infused into the portal vein it induced signalling from the hepatic branch of the vagal afferents and suggests that monitoring of glucose supply to the liver occurs. Hepatic oxidation of fatty acids also induces afferent signalling (Langhans, 1996). The role of lipid and glucose sensors in the liver have been alluded to as separate entities (Mayer 1955; Friedman & Ramirez 1985) but it appears they act synergistically and that altered metabolic signalling would influence nutritional intake (Langhans & Scharrer 1992). This area of investigation has been explored in small animal studies but no significant work has been undertaken in human subjects. Indeed it may well be inappropriate to translate the findings from small animal work to humans where the mechanisms involved in ingestive behaviour are not comparable.

### **Influence of undernutrition on outcome in liver disease**

Although undernutrition is a common feature of chronic liver disease the evidence to support its effect on outcome remains inconclusive. A large (n = 352) longitudinal study on patients with chronic liver disease from the Veterans Administration (Mendenhall, 1986) used eight nutritional indices (percentage ideal weight, triceps skinfold thickness (TSF), mid-arm muscle circumference (AMC), creatinine height index, serum albumin concentration, serum transferrin, total lymphocyte count and delayed cutaneous hypersensitivity) to determine a protein calorie malnutrition score. At time of entry into this study, 8%, 33% and 4% of patients were classified as being mildly, moderately or severely

protein calorie malnourished respectively. Whilst this study highlighted the relationship between severe protein energy malnutrition and mortality, which reached 52% at one month and rose to 76% at a year, it failed to clearly establish a cause and effect relationship between protein calorie malnutrition and disease progression.

Further work which examined the relationship between the degree of undernutrition and disease severity came a decade later when Merli and co-workers (1996) in a five year prospective study of 1053 cirrhotics examined the influence of under-nutrition on mortality among cirrhotics. Patients were stratified for disease severity using the Child's Pugh classification (Table 1.1) (Pugh et al, 1973). This study showed that cumulative survival was lower in patients with depleted muscle mass in Child's A and B groups but not those patients assigned to the grade C group. A low fat mass had no impact on survival in any Child's grade. However, when data were analysed using multivariate analysis; age, sex, bilirubin, cholinesterase, ascites and oesophageal varices were selected as impacting on survival whereas parameters of nutritional status were not. Conversely, in a study of 80 PBC patients, Wicks et al (1995) showed that by using simple anthropometric indices a third of this population could be classified as undernourished and there was an inverse relationship between nutritional status and disease severity. Therefore those patients with the lowest fat mass had a higher Child's Pugh score. Whilst this group observed a significant decrease in fat mass with increasing disease severity, no similar relationship with muscle mass was observed. Although, Wicks et al (1995) did not examine outcome measures their nutritional status data conflicts with that of Merli et al (1996) and suggests there is a different relationship between nutritional status and disease

**TABLE 1.1 CHILD'S PUGH CLASSIFICATION OF CIRRHOSIS**

	Points scored for abnormality			Grade	Scores
Assessment Criteria	1	2	3	Grade	Score
Encephalopathy grade	None	1-2	3-4	A	5-6
Ascites	None	Slight	Moderate		
Bilirubin $\mu\text{mol/l}$	<35	35-50	>50	B	7-9
Biliary cirrhosis	<70	70-170	>170		
Albumin g/l	>35				
Prothrombin time ratio	<1.4	1.4-2.0	>2.0	C	10-15
seconds prolonged	1.4	4-10	>10		

severity in patients with liver disease of hepatocellular and cholestatic origin.

The inability to isolate nutritional status as an independent marker of outcome (Merli et al, 1996) may in part be due to the fact that many so called nutritional indices reflect disease severity rather than nutritional state. For example, Caregaro and colleagues (1996) in a study of patients with hepatocellular disease (alcoholic liver disease  $n = 61$ , viral related cirrhosis  $n = 22$ ) used indices such as retinol binding protein, serum albumin concentration, serum transferrin, skin antigen testing and creatinine height index to determine nutritional status. However, these 'nutritional' markers are strongly influenced by the disease process rather than nutritional state. Not surprisingly these investigators found that anthropometry was the most reliable method of nutritional assessment and whilst they showed poorer survival in patients with low TSF and AMC ( $< 5$ th percentile), they failed to demonstrate a relationship between nutritional status and disease severity.

### **Energy metabolism in liver cirrhosis**

Evidence suggests that the deterioration in body mass found in chronic liver disease may be accounted for by alterations in energy and substrate metabolism as well as changes in nutritional intake. Normally the predominant metabolic pathways in oxidative energy metabolism are the Krebs cycle and  $\beta$ -oxidation. These processes determine metabolic rate which can be measured from the body's heat production or gaseous exchange.

During the latter part of the 19th century, Rubner, a German scientist, built a calorimeter and measured heat production in order to

determine energy production in dogs. Thirty years later Atwater and Benedict (1903) showed that respiratory gas exchange measurements could be used to calculate heat production or metabolic rate in humans. Early calorimetric work was predominantly used in the diagnosis and follow-up of patients with thyroid disease. The majority of these early studies used direct calorimetry to measure energy production. This method involved isolating subjects in an air tight chamber and the heat dissipated by the body was measured by thermo-couples imbedded in the walls of the calorimetry room. As subjects are required to remain for long periods (in excess of 24 hours) direct calorimeters are considered impractical for use in the clinical situation. In addition, operation of the analysers and sensors requires considerable expertise and as a result their use is generally restricted to dedicated nutritional research centres.

Measurement of respiratory gas exchange (oxygen consumption ( $O_2$ ) and carbon dioxide ( $CO_2$ ) production) is termed indirect calorimetry and remains an accepted method of measuring both energy production and substrate utilisation. Indirect calorimetry (open circuit technique) has and continues to be extensively used by metabolic researchers. Vohra and co-workers (1995) and Takala et al (1989) have shown that accuracy and reproducibility in gas exchange results can be obtained when investigators understand the principles of measurement, pay meticulous attention in setting up the equipment and in preparing the subject. A limitation of indirect calorimetry is that short periods of calorimetry (i.e. 40 min) are extrapolated to 24 hour periods and preclude study of diurnal effects.

The oxidation of substrates (carbohydrate, fat, protein and alcohol) to yield energy involves the consumption of  $O_2$  and production of  $CO_2$

and water ( $\text{H}_2\text{O}$ ). Each substrate consumes varying quantities of  $\text{O}_2$  and the energy yield per gram of fat, carbohydrate, protein and alcohol are: 39.63 kJ (fat), 15.56 kJ (glucose), 17.50 kJ (starch) and 19.68 kJ (metabolisable energy from protein) and alcohol 29.67 kJ (ethanol) respectively. Fat the richest source of energy yields more than twice the energy from carbohydrate or protein. Some of the oxygen and carbon of amino acids remain combined with nitrogen and is excreted as nitrogenous products in the urine and to a lesser extent in the faeces. The contribution of protein oxidation to the total  $\text{O}_2$  and  $\text{CO}_2$  can be estimated by taking into account the urinary excretion of nitrogen.

Energy expenditure can be derived from gaseous exchange and nitrogen excretion and one of the most widely used equations is that of Weir (1949) and is based on measurements of changes in oxygen content of inspired and expired air. Gas exchange measurements can also be used to determine substrate oxidation (Elia 1992; Frayn 1983, Acheson et al, 1984). The ratio of expired  $\text{CO}_2$  to consumed  $\text{O}_2$  is termed the respiratory quotient (RQ) where the  $\text{O}_2$  consumption and  $\text{CO}_2$  production differs with each macronutrient oxidised. For example, following complete oxidation of one molecule of glucose, 6 mols of  $\text{O}_2$  are consumed and 6 mols of  $\text{CO}_2$  are produced and the RQ ( $\text{CO}_2 : \text{O}_2$ ) of carbohydrate is 1.0.

Measurement of urinary nitrogen excretion over a study period allows calculation of the non-protein respiratory quotient (NPRQ). The conversion of total RQ to NPRQ has been shown to represent only a small change in oxidation rates and as a result is often ignored in metabolic calculations.

In summary, indirect calorimetry is a useful technique which can be used to measure energy production and substrate oxidation in human

subjects so long as its limitations are fully understood (Webber and Macdonald, 1995).

### **Energy Expenditure in Liver Cirrhosis**

The largest component of total energy expenditure is resting energy expenditure (REE) accounting for 60 - 70% of energy output. Other components include physical activity (15-30%), diet induced thermogenesis (7-13%) and other factors (e.g. smoking, effects of drugs, thermoregulation (2-7%) (Elia 1992). The lean body mass (LBM) a term synonymously used with fat free mass (FFM) contains the metabolically active component of body composition and as such is closely related to REE.

Several energy predictive formulae use the variables of age, sex, height and weight to estimate REE and include those of Harris-Benedict (1919), Kleiber (1932), Fleisch Tables (1951), Robertson-Reid (1952) and Schofield (1985). These predictive formulae were derived from direct calorimetry studies performed on healthy subjects. The later work of Schofield (1985) was derived from a review of predictive data on 7,173 subjects from various nationalities and also included a number of underweight individuals. The Schofield (1985) equation may therefore be more applicable to the clinical situation. In a number of disease states there may be alterations in body composition and estimating REE from predictive formulae derived from healthy populations may provide erroneous results by over or under-estimating the size of the metabolic component of body composition.

In chronic liver disease changes in body composition may be reflected in a decline in fat and muscle mass (Wicks et al 1995) but the



presence of fluid retention in some patients makes it difficult to predict the energy expenditure of this population.

In light of the problems in estimating the size of the metabolically active component of body composition and the heterogeneity of patients with chronic liver disease, the energy cost of this pathology remains one of debate. Nevertheless, the importance of elucidating the energy requirements in liver disease should not be underestimated where overfeeding could result in metabolic abnormalities and underfeeding exacerbate undernutrition. Indeed, clarification of energy requirements in liver disease would permit clinicians and nutritionists to establish and meet the nutritional needs of their patients.

Several studies have suggested that liver disease exerts a hypermetabolic effect (Shanbhogue et al 1987; John et al 1989; Schneeweiss et al 1990; Muller et al 1991; Muller et al 1994). Conversely, the work of Owen et al (1983), Jhangiani et al (1986) and Merli et al (1990) suggest that energy expenditure in liver cirrhosis is not significantly different from healthy control subjects. A number of criticisms can be leveled at these studies, many were carried out on small numbers of patients and REE results were not stratified for pathology or disease severity. In addition the approach to expressing energy results varied; some used the relationship of REE to body surface area (Jhangiani et al 1986; Shanbhogue et al 1987), others the relationship of REE to LBM derived from urinary creatinine excretion (Di Cecco et al 1989; Nielsen et al 1993) or lean body mass derived by bioelectrical impedance analysis (BIA) (Muller et al 1991).

For example, a study by Shanbhogue et al (1987) measured REE by indirect calorimetry in a heterogeneous group of ten patients with end



stage liver disease and ten healthy controls. In this study urinary creatinine excretion was used as an index of LBM and results showed that energy expenditure in patients with liver disease when compared with control subjects was significantly higher (cirrhotic patients:  $1900 \pm 610$  kcal/g creatinine/day versus controls:  $1180 \pm 260$  kcal/g/creatinine/day). Similarly John and colleagues (1989) in a study of 20 patients with alcoholic hepatitis (sub-divided into moderate and severe hepatitis) and control subjects observed no differences in REE when severity of disease was considered. However, when REE was related to LBM (estimated by urinary creatinine excretion) the energy expenditure of patients with severe alcoholic hepatitis was 55% greater than control subjects (cirrhotic patients: 1813 kcal/g creatinine/day versus controls: 1043 kcal/g creatinine/day).

The presence of hypermetabolism in liver disease is also supported by the work of Schneeweiss and co-workers (1990) who studied 35 patients with acute hepatitis, 22 patients with alcoholic cirrhosis and 20 healthy controls. When compared with the control group they found no appreciable alterations in energy metabolism among patients with acute hepatitis whereas those with alcoholic cirrhosis were hypermetabolic (cirrhotic patients: 1657 kcal/g creatinine/day versus controls: 1007 kcal/g creatinine/day). In patients where alcoholic hepatitis and cirrhosis co-exist, this could add to the metabolic cost of the disease and account for the observed hypermetabolism. However, Schneeweiss et al (1990) also stratified these patients using the modified Child's Pugh score (Pugh et al 1973) and noted an inverse relationship between energy expenditure and severity of disease, in other words the greater the severity of disease the lower the REE. This finding is at odds with the hypermetabolism seen in

their patients with alcohol cirrhosis and may in part be explained by a reduction in oxygen requirements as a result of a decrease in the size of the body's metabolically active tissue.

In addition, Moreau et al (1988) used haemodynamic studies to determine oxygen uptake and transport in 35 patients with alcoholic liver disease graded for disease severity (Pugh et al 1973) and observed a progressive reduction in  $O_2$  uptake. These findings are at odds with those of Green et al (1991) who observed a positive relationship between oxygen consumption and disease severity in seven PBC patients suggesting that as liver disease progresses there is a concomitant increase in oxygen consumption and REE. The contradictory findings of these two studies are difficult to reconcile but it may be that differing pathologies elicit different metabolic responses. In addition, investigators who classified patients with liver disease as hypermetabolic using creatinine excretion may be using an inappropriate marker of lean tissue. Urinary creatinine excretion is used to reflect muscle mass which accounts for approximately half the lean tissue in healthy adults. However, the origin of endogenous creatinine is from the synthesis of its precursor creatine found in the liver and kidney. In health, creatinine is produced non-enzymatically from creatine at a constant daily rate. Although there is considerable intra individual variability in daily urinary creatinine excretion (11- 30%) (Greenblatt et al, 1976, Lykken et al 1980) it has been shown to significantly correlate with muscle mass and LBM when compared with  $N_{15}$  isotope dilution techniques (Heymsfield et al, 1983). However, in wasting diseases such as cancer and chronic liver disease, the ratio of skeletal muscle mass to other metabolically active organs may alter where a loss of muscle mass would increase the proportion of LBM

from the metabolic organs (liver, heart and brain) and would result in an inappropriately high energy expenditure result. Finally, as creatine is synthesised in the liver, in cirrhosis there may be alterations in synthesis and the use of creatinine as a marker of lean body mass in liver disease seems inappropriate.

Whilst some investigators have suggested liver disease is a hypermetabolic state, others such as Owen and colleagues (1983) in a study of nine patients with alcoholic liver disease, Merli et al (1990) in a study of 25 cirrhotics and Jhangiani et al (1986) in a study of eight ALD patients have shown their patients to be normometabolic.

There appears to be no consensus as to the energy cost, if any, of chronic liver disease. Indeed, in one of the largest studies in cirrhotic patients (n = 123), Muller and colleagues (1994) reported that 18%, 52% and 31% could be classified as hypometabolic, normometabolic and hypermetabolic respectively. Difficulties in measuring energy status in some patients with liver disease are due to problems in determining the size of the metabolically active tissue. In order to further knowledge of energy metabolism in liver disease, larger more focused studies are required that would permit the stratification of patients by pathology e.g. alcoholic cirrhosis, primary biliary cirrhosis and/or the severity of disease. In addition, longitudinal nutritional studies of this population could yield important data on the progression of disease and its effect on metabolism.

### **Energy Expenditure following Liver Transplantation**

Only two studies have examined energy expenditure following orthotopic liver transplantation (OLT). Green et al (1991), in a small (n = 7) homogeneous cross sectional study of patients with PBC, compared

energy metabolism pre- and post-OLT. When compared with OLT patients and the age and sex matched control group, the PBC group were hypermetabolic (PBC 4.64 kJ/hr/kg body weight, OLT 3.39 kJ/hr/kg body weight and controls 3.65 kJ/hr/kg body weight). This group studied a different cohort before and after OLT and thereby weaken their findings as the intervariability of energy expenditure are considerable ( $\pm 15\%$ ) (Webber & MacDonald 1995). In a longitudinal energy expenditure study of 26 cirrhotic patients subjected to OLT and reviewed 432 days after surgery (range 103-1022 days) it was suggested that patients who were hypermetabolic pre-operatively were more likely to develop postoperative complications (e.g. rejection, infection) (Muller et al 1994). This study has provided the first evidence to suggest that the presence of hypermetabolism before transplantation is associated with a poor postoperative prognosis. These investigators suggest preoperative hypermetabolism persists for up to three years after OLT. However, this finding is difficult to understand in the uncomplicated OLT patient where the presence of an ongoing inflammatory response of three years duration seems unlikely.

This literature review of studies carried out in patients with chronic liver disease highlights the difficulties in finding practical, reproducible and specific indices of body composition that are related to REE. In cirrhotic patients with ascites or peripheral oedema, significant expansion of the extracellular water (ECW) space means that estimates of body mass and four site skinfold measurements may yield erroneous results.

The difficulties of obtaining practical and accurate methods of body composition in liver disease was addressed by Muller and colleagues in 1992. This group used single frequency bioelectrical impedance analysis

(BIA) to determine LBM in ten patients with alcoholic liver disease. They previously validated the use of BIA in 17 patients pre- and post-paracentesis and compared these results with BCM derived from total body potassium measurements ( $r=0.84$ ,  $p<0.001$ ) and noted that their ALD patients were hypermetabolic. In this study the relationship between total body potassium (TBK) and LBM estimated by BIA remained significant even when used among patients with moderate ascites. This group used BIA equations derived from isotope dilution studies in a heterogeneous surgical population which included cancer patients where expansion of the extracellular water compartment is common (Fearon et al 1992).

### **Bioelectrical Impedance Analysis**

Bioelectrical impedance analysis has been used extensively in the determination of body composition. Impedance analysis does not require any specific expertise, is non-invasive and therefore acceptable to the patient. Bioelectrical impedance measures tissue conductivity where the conductivity of a region of the body is directly proportional to the amount of electrolyte rich fluid present. It has been suggested that BIA can be used to measure total body water (TBW) from which LBM may be estimated and then by subtracting LBM from body weight, fat mass may be derived. In the healthy individual, these measurements can provide accurate determinations of body composition (Kushner & Schoeller, 1986; Lukaski, 1986). Although there is some debate as to the value of the technique in the clinical situation, where in disease (e.g. cancer, critical illness) there may be fluid shifts. This may be generalised such as in peripheral oedema or may be regional as in ascites.

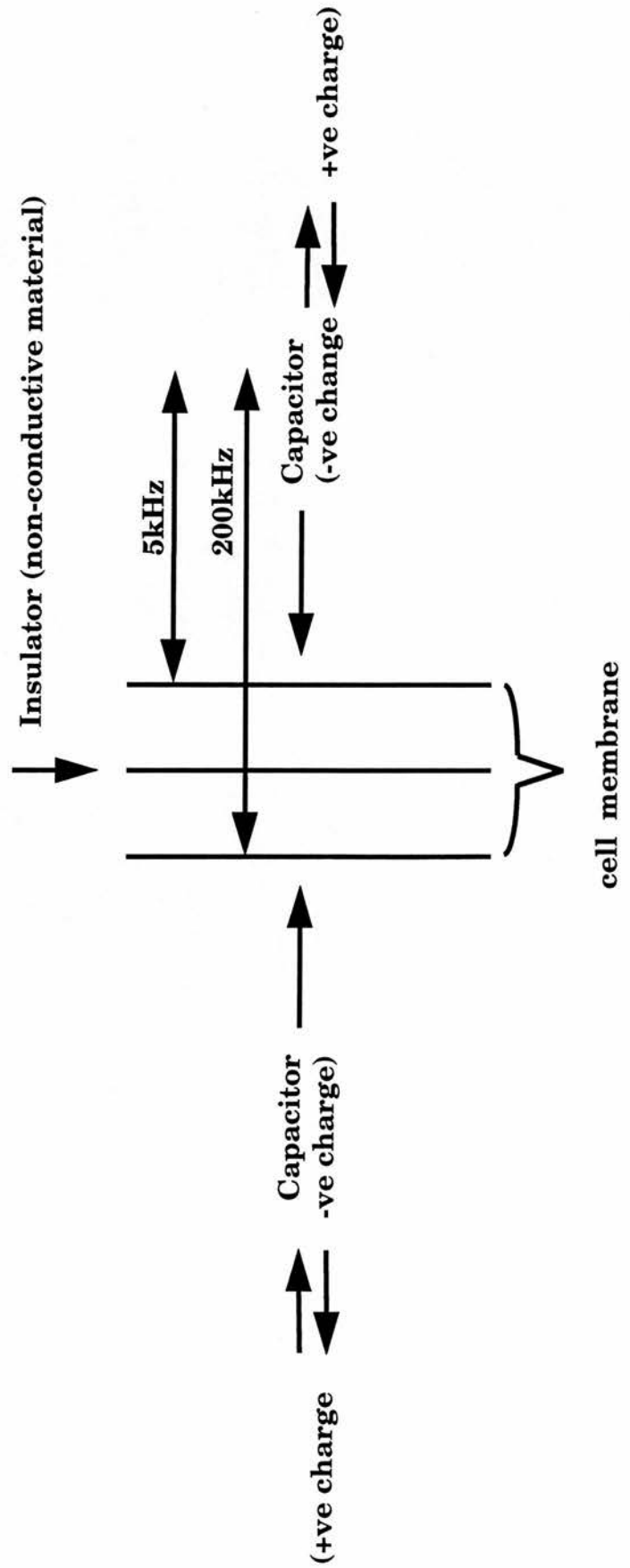
Before considering the effect of altered fluid distribution on BIA, there are a number of other factors that could influence impedance results and may account for some of the disparity found in the literature. These include:

- a. differences in the type of analyser used and the operation frequency (e.g. single frequency, dual frequency or bioimpedance spectroscopy).
- b. lack of standardisation of:
  - i. skin preparation
  - ii. site of electrode placement
- c. equations used in the calculation of total body water and extra-cellular water; use of the manufacturers equations are generally derived from a healthy population and are inappropriate when used in the clinical situation.
- d. positioning of the subject prior to and the timing of BIA measurements.
- e. recent food/fluid intake.

With BIA, the conductivity of the body's tissue is determined by passing a small alternating (+ve and -ve reversing charge) electric current through the body. The greater the frequency, the faster the current will alternate between positive and negative charges (Figure 1.2). At lower frequencies (5 kHz), the current is unable to penetrate the cell membranes and will therefore estimate ECW whereas at higher frequencies (200 kHz) the current can penetrate cell membranes and permit estimation of TBW. Intra-cellular water (ICW) may be estimated by subtracting ECW from TBW. From ICW body cell mass (BCM) can be determined (Shizgal, 1983) and is the metabolically active component of the body. The body

**FIGURE 1.2**

**SCHEMATIC REPRESENTATION OF MODE OF ACTION OF BIA WHERE INCREASING  
FREQUENCY ALLOWS CURRENT TO PASS THROUGH CELL MEMBRANE**





cell mass is the oxygen consuming, carbon dioxide producing, work performing tissue and as such will dictate energy expenditure (Moore, 1980). A reference method used in determining body cell mass is total body potassium measurements but few hospitals have access to whole body monitors. In healthy adults the precision of whole body counting is 2-5% (Cohn & Parr 1985) and this method provides the best index of body cell mass where:

$$\text{BCM (kg)} = 0.00833 \times K \text{ (mEq)}$$

In order to validate the use of impedance in the clinical situation, a number of researchers have compared reference method data with BIA results (Lukaski, 1996). Isotopic techniques are the most commonly used reference methods for determination of TBW and ECW where deuterium and radiobromide are used to assess LBM and BCM respectively. The precision of these isotopic techniques is described as being 1-2% for TBW and 2-5% for ECW (Schoeller 1996). In patients with fluid retention, particularly those with severe ascites, accuracy of isotopic techniques may be affected by difficulties in determining the time to isotope equilibrium (normally 4 hours for deuterium and 18 hours radiobromide).

Several studies have shown a strong correlation between single frequency BIA (50 kHz), isotope dilution and densitometric determination of TBW and LBM (Kushner & Schoeller 1986; Lukaski 1996). Early work from our own unit (Fearon et al, 1992) used single frequency BIA among a heterogeneous surgical population (neoplastic disease, irritable bowel disease and pancreatitis) and compared BIA results with isotope studies (tritiated water, sodium bromide and TBK) to determine TBW, ECW and BCM. In the 43 patients studied, there was a significant relationship between BIA and body habitus measurements for tritium ( $r^2 = 0.862$ );



total body potassium ( $n = 31$ ) ( $r^2 = 0.948$ ). Although BIA tended to underestimate TBW, the standard error of estimate (SEE) was 2.7 litres and the co-efficient of variation (CoV) was 8.1% in patients compared with 3.4% for healthy subjects (Lukaski et al, 1986). This suggests that the greater error found in patients is related to changes in fluid and electrolyte composition in disease which may alter conductivity of body compartments. However, when total body potassium was considered the SEE was 6.3 g and CoV of 6.4% and suggests BIA provides a useful index of body cell mass. Whilst single frequency BIA cannot detect small changes in body composition, it does provide a useful method of defining the characteristics of patient populations or as a method to examine large changes in body composition in longitudinal studies.

Patients with liver disease may have alterations in fluid distribution and the non-invasive and simplistic nature of BIA has, as expected, attracted considerable interest amongst specialists in liver disease. However, studies in the use of single frequency BIA in liver disease have been variable and a criticism of some studies is that many investigators used manufacturers equations based only on body habitus measurements. These equations were originally derived from a healthy population and their application seems inappropriate among patients with chronic liver disease. For example, Prijatmoko et al (1993), in a study of 38 male alcoholic patients, used BIA (50 kHz) and compared results with a number of body composition reference methods (dual X-ray absorptometry, deuterated water, total body nitrogen). In this study patients were sub-divided by Child's Pugh classification and the presence of ascites was not considered. Not surprisingly this group found that BIA was not a useful predictor of body composition.

Guglielmi et al (1991), in a study in male cirrhotic patients, considered the use of single frequency BIA among 36 and 22 non-ascitic and ascitic patients respectively. This group compared whole body resistance between age and sex matched controls to patients with and without ascites. As expected, patients with ascites had a lower whole body resistance when compared to patients without ascites or control subjects and the lower resistance value suggests that ascitic patients have higher total body water. An important finding in this study was the increased error seen in patients with significant ascites where the ascitic patient's CoV varied substantially over three days (2.2% controls, without ascites 2.4%, with ascites 11.3%) and suggests that single frequency BIA cannot be used to determine body water in patients with significant ascites.

The reason for the inability of BIA to determine the regional accumulation of fluid may be that single frequency BIA has limitations in determining TBW as 50 kHz cannot penetrate cell membranes. Additionally, tetra polar BIA measurements are made from the hand and foot, the contribution from the trunk to whole body impedance is approximately 10% (Foster & Lukaski 1996).

It is possible that improvement in the error of prediction for TBW could be obtained by using higher frequencies that pass through the intracellular space. Indeed several manufacturers have developed multi-frequency bioelectrical impedance analysers (MFBIA) that operate from 1kHz to 1MHz.

Early work on MFBIA by Segal and colleagues (1991) used three frequencies (5, 50, 100 kHz) to estimate ECW and TBW in healthy males (n = 36) and compared these results with isotopic studies. This group found

that when compared to isotope determination the optimum frequencies for determining ECW was 5 kHz and for TBW 100 kHz. A logical progression from this work was to examine the validity of MFBIA in disease. Hannan et al (1995), in a study of surgical patients with varying pathologies, showed that when compared with isotopic studies (radiobromide, tritium) using frequencies of 5 and 200 kHz the technique provided a useful estimate of ECW (5 kHz) and TBW (200 kHz). These frequencies provided similar errors when compared with the more complex use of bioelectrical spectroscopy where resistance and reactance were measured at a range of frequencies from 5 kHz to 1 MHz. The results of spectroscopy gave mean CoV for ECW and TBW of 0.9% and 3% respectively whereas for MFBIA at 5 and 200 kHz the corresponding CoV of variation were 0.9% and 0.6%. This study concluded that an impedance analyser operating at 5 and 200 kHz compared favourably with the more complex technique of bioelectrical spectroscopy in body composition determination.

Cha and co-workers (1995) used impedance among patients with end stage renal disease and found that the impedance index ( $\text{height}^2/\text{resistance}$ ) at 5 kHz correlated most closely with ECW determined by radiobromide dilution. Total body water measured by deuterium showed the strongest correlation with resistance measurements at 500 kHz ( $r = 0.974$ ). This study provides evidence that MFBIA may assist in the evaluation of fluid distribution and body composition in patients with clinical disease.

Bioelectrical impedance compared with neutron activation studies by Muller et al, 1991, 1994) in liver disease has shown that in patients with abdominal ascites of up to 4 litres, BIA can provide a useful insight into

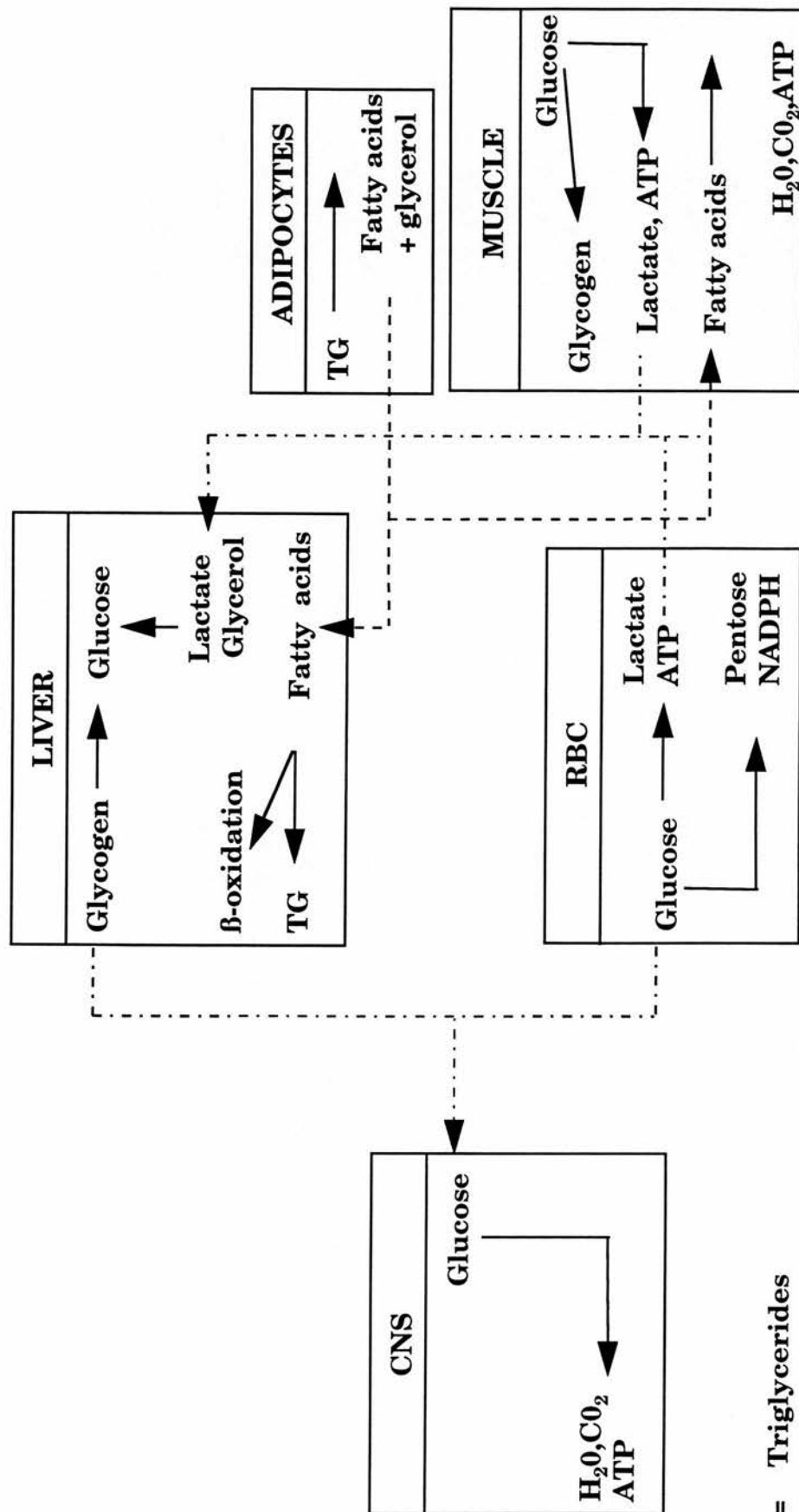
body morphology. It is important, however, that in using BIA the investigator appreciates factors that will affect the reproducibility of impedance measurements. In addition, a component of the prediction errors associated with impedance may in fact be attributed to the reference methods used. For example, when considering radioisotope dilution it is often difficult to determine the reproducibility of these methods in surgical patients because of the relatively long half life of such isotopes. Hannan et al (1994) suggested that repeated measurements of TBW in a control population indicates that a component of prediction error, which can be attributed to radioisotope dilution is significant. Despite its limitations, the use of MFBIA provides useful information on body composition in patients where reference methodology is inaccessible.

### **The Liver and Substrate Metabolism**

The liver is responsible for the flow of substrates between organs and tissues and in response to humoral and neural input maintains fuel homeostasis. The metabolic pathways of glucose, lipid and protein are interdependent and whilst they may be examined individually, they must also be considered as an entity where the synthesis and breakdown of macronutrients is inextricably linked (Figure 1.3).

Metabolic sensors detect circulating levels of macronutrients and depending on the body's immediate energy requirements directs specific macronutrients for breakdown (e.g. glycolysis) or storage (e.g. glycogenesis, lipogenesis). These activities are primarily directed by the nervous, endocrine and vascular systems. In times of long term fasting

**FIGURE 1.3** POST ABSORPTIVE FLOW OF SUBSTRATES BETWEEN THE LIVER, CNS, ADIPOSE TISSUE, MUSCLE AND RED BLOOD CELLS



TG = Triglycerides

ATP = adenosine tri-phosphate

Adapted from Frayn, 1997

the liver's energy is derived from adipose tissue (fatty acids and glycerol), skeletal muscle (lactate and pyruvate) as well as alpha-ketoacids produced by transamination of the branch chain amino acids (leucine, isoleucine and valine).

The synthesis, transport and breakdown of macronutrients is predominantly orchestrated by the endocrine system and is related to the quantity and quality of dietary intake, post-absorptive time as well as the availability of glucose in relation to the requirements of the body's glucose oxidising tissues. For example, during short periods of nutrient deprivation, the liver maintains glucose homeostasis by glycogenolysis and gluconeogenesis primarily directed by glucagon. However, glycogen stores are limited and are normally exhausted after a 48 hours fast and thereafter greater reliance is placed on glucose derived from skeletal muscle breakdown (e.g. lactate, alanine), glycerol and free fatty acids from lipolysis. In prolonged fasting (>72 hours) the body, in an attempt to preserve LBM reduces the requirement for glucose by increasing the energy derived from endogenous fat reserves.

Conversely, in the fed state, the glucose not immediately required for energy will be converted to glycogen but once stores are replete excess glucose will be directed as fatty acids to the adipocytes. In addition, dispensable amino acids (alanine, glutamate, aspartate) are synthesised in the liver and are directed to skeletal muscle for incorporation into muscle proteins.

This brief overview of the liver's role in macronutrient metabolism has laid the foundations for an appreciation of alterations in substrate metabolism that may occur in chronic liver disease and effect changes in nutritional intake and nutritional status.

## Substrate Metabolism in Liver Disease

In chronic liver disease rates of substrate oxidation are altered, where in the post-absorptive state there is an increase in lipid oxidation and a decrease in glucose oxidation (Owen et al, 1983; Nosadini et al, 1984; Merli et al, 1990; Muller et al, 1991; Green et al, 1991; Campillo et al, 1992; Muller et al, 1994). However, the methodological approaches used by these research groups to determine substrate oxidation rates warrants further discussion.

Owen et al (1983) examined the type and quantity of substrate oxidised in eight ALD patients and four unmatched controls. Fatty acid oxidation and turnover was measured using indirect calorimetry and simultaneous  $^{14}\text{C}$ -tracer analysis ( $^{14}\text{C}$  palmitate). The most important finding was that the metabolic profile observed in these patients after an overnight fast was indicative of a more extended period of fasting (48-72 hours) (cirrhotic patients  $\text{RQ} = 0.74 \pm 0.02$  compared with controls  $\text{RQ} = 0.88 \pm 0.01$ ). This phenomenon could be explained by the diminished availability of hepatic glycogen. In alcoholic liver disease glycogenolysis accounts for approximately 33% of glucose production and gluconeogenesis 67% (Owen et al 1981). This is a twofold increase in gluconeogenesis when compared with controls. If the increased demand for glucose derived from skeletal muscle mass (gluconeogenesis) continued for a prolonged period it would contribute to a deterioration in nutritional status. In addition, a prolonged up-regulation of the gluconeogenic pathway would increase dietary protein requirements (Kondrup and Muller, 1997). However, Merli et al (1990) in a study of 25 patients with liver disease (heterogeneous) reported that protein oxidation rates and urinary nitrogen excretion were reduced and reflected



simple starvation. It may be that increased lipolysis and concomitant reliance on fat oxidation could be an adaptive response which acts to preserve lean tissue.

Alterations in fasting substrate metabolism are mediated by the endocrine and sympathetic nervous system (SNS), the influence of which may be disturbed in liver disease. The effect of chronic liver disease on hormonal systems is one of debate although hyperinsulinaemia and hyperglucagonaemia are features of advanced liver disease (Berkowitz, 1969; Bosch et al, 1984; Kabadi et al, 1984; Taylor et al, 1985; Silva et al, 1988). A study by Romijn and colleagues (1991) extended the pioneering work of Owen et al (1983) and examined glucose and lipid metabolism after a 16 and 22 hour fast in 16 cirrhotics patients. Following a 16 hour fast results were comparable with other studies (Owen 1983; Nosadini et al, 1984; Muller et al, 1992) and showed that in the pre- and post-absorptive period lipid is the body's predominant energy substrate. However, the results of the 22 hour fast are of particular interest in that an extension of the fast by 6 hours depressed glucose oxidation in controls but this effect was even more pronounced in cirrhotic patients. This decrease in glucose oxidation may be explained by the liver's deteriorating function and inability to maintain adequate glycogen reserves.

The mechanisms underlying the elevation of lipolysis in cirrhosis have been considered. For example, catecholamines are implicated in the regulation of lipolysis and elevated concentrations of adrenaline have been recorded in patients with cirrhosis (Campillo et al, 1992). However, Romijn et al (1991) observed no difference in fasting concentrations of catecholamines or insulin following a 16 or 22 hour period of fasting in



cirrhotic patients or controls. Although, elevated glucagon concentrations were observed in cirrhotic patients after the 22 hour fast and this increase would augment lipolysis. Although not a universal finding, elevated glucagon concentrations in liver disease should act to promote gluconeogenesis from extrahepatic sites.

It has been suggested that the hyperinsulinaemia observed in chronic liver disease is unrelated to increased stimulation of pancreatic beta cells (Greco et al, 1979). This hyperinsulinaemia but may be accounted for by deficient hepatic insulin extraction or to the shunting of portal venous blood to the systemic circulation thereby bypassing the liver (Nolte et al, 1995). Animal studies that support the role of shunting in cirrhotic hyperinsulinaemia have observed elevated insulin concentrations after construction of portacaval shunts (Tranberg et al, 1979). However, other researchers have failed to attribute the hyperinsulinaemia of cirrhosis to portacaval shunting (Smith-Laing et al, 1979).

Taylor et al (1985) and Cavallo-Perin et al (1985) suggest that the hyperinsulinaemia of cirrhosis is a result of a deficit in binding and post-binding defects in insulin target organ cells such as adipocytes. Following an oral glucose tolerance test, Taylor et al (1985) assessed insulin sensitivity in 16 cirrhotics and 11 controls. The cirrhotic patients had an impaired glucose response to the glucose tolerance test despite elevations in insulin secretion when compared with control subjects. In addition, Taylor et al (1985) also observed differences in the responses to the glucose tolerance test two hours after ingestion in patients with alcoholic liver cirrhosis, biliary cirrhosis and cryptogenic cirrhosis. Blood glucose and serum insulin concentrations were significantly higher in alcoholic



patients when compared to those with biliary cirrhosis. This finding suggests that the response to a glucose load will differ depending on the primary aetiology of cirrhosis. It could be postulated that the improved glucose and insulin response to the glucose challenge is because hepatocyte function is better preserved in PBC.

In an attempt to define the response to feeding in cirrhosis, only a few groups have examined substrate oxidation in the fasted and fed state (Eriksson et al, 1989; Green et al, 1991; Campillo et al, 1992). These studies considered small numbers of patients and with the exception of the work of Green et al (1991) focused on patients with alcoholic liver disease. These investigators found that, in the fasted state, lipid oxidation rates were elevated in cirrhotic patients when compared with control subjects. However, following consumption of a test meal, Eriksson et al (1989) and Campillo et al (1992) observed significantly enhanced glucose oxidation in their alcoholic cirrhotic patients when compared with controls. On the other hand in a study of PBC patients Green et al (1991) observed no significant differences in glucose oxidation in the transition from the fasted to the fed state. This is indicative of differential nutrient handling in patients with alcoholic and cholestatic disease. It is, however, difficult to make direct comparisons between these studies where the size of test meals ranged from 41 kJ/kg body weight to a prescriptive meal of 4 MJ. In addition the duration subjects were studied for ranged from 3-6 hours postprandially.

Further well controlled studies are required where the length of study is sufficient to observe the peak and duration of the response to feeding yet short enough to be acceptable to the patient.

## **Effect of Liver Transplantation on Metabolic Status**

Little more than a decade ago OLT was considered by many clinicians to be a dangerous and an almost experimental procedure. In 1980 less than 50 grafts were performed across Europe whereas in 1997, 650 liver transplants were undertaken in the United Kingdom. Outcome has improved too and in the early 1980's only one patient in three could expect to survive to one year whereas only one patient out of four does not survive to one year in the 1990's (McMaster, 1994). This improved survival has been associated with improvement in the quality of donor organs, improved organ perfusion and the introduction of the immunosuppressive drug cyclosporin (CsA). In order to maximise pre- and post-transplant care, all patients are regularly assessed, evaluated and monitored by a highly trained multidisciplinary team (surgeon, physician, nurse, clinical biochemist, physiotherapist, psychiatrist, pharmacist and dietitian). The transplanted patient's long term management is dependent on their pharmaceutical regimen where the balance of immunosuppression is crucial. Too low a dose increases the risk of rejection and too high a dose could induce toxic effects such as nephrotoxicity.

Common metabolic problems that occur following transplantation include diabetes, hyperlipidaemia, hypertension and obesity. The aetiology of these disorders has predominantly been attributed to immunosuppressive therapy which is generally given as CsA or tacrolimus monotherapy, or in combination with prednisolone and/or azathioprine. Normally steroids are reduced during the first three months following surgery and may be discontinued shortly thereafter.

The drug regimen will, of course, be dictated by individual patient progress.

As the number of surviving longterm transplant recipients increases, several research groups have examined the prevalence of the metabolic abnormalities which frequently occur in liver grafted patients. For example, a number of patients not previously diabetic (DM) exhibit a DM oral glucose tolerance test although evidence suggests this diabetes is transient in nature. In a retrospective review of 88 OLT patients (none with a previous DM history), Navasa et al (1996) noted the prevalence of diabetes post-transplant was 27%, 9% and 7% at one, two and three years respectively. Furthermore, Tabasco-Minguillan et al (1993) considered the diabetogenic effect of the immunosuppressive drug tacrolimus and during the early postoperative period, seven out of 52 patients became DM but at a year OLT only one patient required insulin and this was discontinued by 18 months.

Another condition that could indirectly influence longterm survival in liver transplantation is hyperlipidaemia. Accelerated atherosclerosis has been observed in patients receiving solid organ transplants and would increase cardiovascular risk (Munoz et al, 1991; Kasiske 1988). Kasiske (1988) in a retrospective study of 403 renal transplants found that 15% of patients developed ischaemic heart disease. Interestingly, age, male sex, diabetes, smoking, hypertension and plasma cholesterol were independently associated with post-transplant vascular disease. Over the next eight years, Kasiske and colleagues (1996) used drugs to treat these lipid abnormalities in transplanted patients and on re-examination they found that cholesterol was no longer a predictive factor of ischaemic heart disease. Whilst no conclusive evidence exists, CsA has been implicated in

the pathogenesis of hyperlipidaemia, although concomitant steroid therapy can also adversely effect lipid metabolism in recipients of solid organ transplants (Becker et al, 1988).

The detrimental influence of CsA on plasma lipids was examined by Ballantyne (1989) who showed that patients treated with CsA for psoriasis or amyotrophic lateral sclerosis had elevated plasma lipids following treatment. It has been suggested that the newer immunosuppressive drug tacrolimus may improve the lipid profile of transplanted patients. Indeed Van Thiel et al (1990) has shown that tacrolimus administration after OLT did not significantly elevate serum cholesterol levels.

One distressing, common and long term post-operative feature of OLT is that of progressive and significant weight gain. This seems somewhat of a paradox since many patients lose weight before transplant and are frequently prescribed energy dense dietary regimens. The incidence of obesity following OLT has been reported by several groups and ranges from 40-64% (Stegall et al, 1995; Munoz et al, 1991; Palmer et al, 1991). Munoz et al (1991) studied 21 OLT patients for more than 18 months following transplantation and noted that those patients who became overweight or obese were not different in terms of age, sex, length of postoperative stay, serum cholesterol or CsA levels than their weight stable counterparts. Interestingly, this study is one of the few that examined energy and macronutrient intake following transplantation (18 months OLT). They found no significant differences in dietary intake between OLT patients and an age and sex matched control group. Unfortunately, these important data received little attention by the authors but it would appear a three day dietary record was kept by

patients but only on one occasion. Sequential monitoring of dietary intake and its relationship with weight gain would contribute to understanding the mechanisms involved in OLT weight gain.

Stegall et al (1995) examined weight status in 123 patients one year OLT and found that 39% of men and 42% of women had a body mass index ( $BMI \geq 27$ ) and could be classified as being overweight. In this study the tapering of steroids to 5 mg/day significantly reduced the prevalence of diabetes, hypertension and hypercholesterolaemia but there was no change in the prevalence of obesity. Palmer and co-workers (1991) studied weight gain in 28 patients pre- and post OLT and found that two-thirds of these patients became overweight (64%) and this occurred during the second to sixteenth month after OLT. Interestingly in the study of Palmer et al (1991), all patients who were overweight before OLT became significantly more overweight after their graft. The rate of weight gain among the overweight population was 1.5 kg/month compared with 0.4 kg/month in the non-overweight group. These workers attributed weight gain to the hyperphagic effect of prednisolone. However, this seems unlikely as no differences in prescribed drug regimen in the overweight and non-overweight group were apparent and does not account for the three-fold increase in monthly weight gain found only in the pre-OLT overweight population. The findings of this study suggest that the effect of low prednisolone doses on weight gain may have been overstated particularly in view of the unique role of the liver as a metabolic sensor (Friedman, 1997). In addition, the increase in appetite induced by steroids may be transient and does not not necessarily reflect qualitative intake (Bruera et al, 1990).



A post-OLT period of rapid weight gain could contribute to increased cardiovascular morbidity and mortality. Furthermore, a significant increase in body weight among patients transplanted for PBC could make them even more prone to pathologic fractures. These debilitating complications contradict the philosophy of transplant surgery which is to enable recipients to return to a life of normal activity. Whilst the prevalence of rapid weight gain post-OLT has been reported, body composition changes and the mechanisms involved in weight gain following liver transplantation remain poorly understood.

### **Dietary Intake**

A number of methods may be employed to estimate quantitatively and qualitatively dietary intake. These include the weighed intake technique, estimated records, dietary history and food frequency questionnaires (Gibson, 1990). Whilst the weighed record technique is considered the most accurate method of quantifying dietary intake, this approach requires a high level of subject co-operation as every component of the diet must be accurately weighed and meticulously recorded by the subject, carer or investigator (Pekkarinen 1970). This approach in estimating dietary intake would prove too demanding for patients with chronic disease and a more acceptable yet valid method of estimating nutritional intake may be obtained from the diet diary method.

To obtain valid results of dietary intake it is essential that subjects are carefully instructed on how to complete their diet diary where detailed descriptions of all food and beverages (including brand names and composite dishes) consumed and cooking methods used, is documented. When using this technique, the investigator must be aware of sources of

measurement error such as subject bias, and although over- or under-reporting has been observed, this has primarily been among individuals with eating disorders (Goldberg et al, 1991; MacDiarmid and Blundell, 1997; Poppitt et al, 1998). The subject may also incorrectly estimate the portion sizes of food consumed and with regard to the investigator, coding and computer errors may occur. However, these errors can be minimised by carefully instructing subjects on correct recording of food portion sizes and in the use of household measures. Additionally a structured dietary record proforma which uses appropriate language will act to review and reinforce the information given at interview.

The information from three day diet diaries will provide an estimate of energy and macronutrient intake (Gibson 1990). Nettleton et al (1980) in a study of 38 subjects showed that when estimated intake, using household measures, was compared with weighed intake, the percentage of subjects whose intake was within  $\pm 10\%$  was 92% for energy, 87% for protein, 71% for fat and 89% for carbohydrate.

The number of days a dietary diary is kept will affect the precision of the method. It must be long enough to provide valid information but at the same time not too arduous for the population being studied. Bingham et al (1982) observed that in a sample size of 500, a 68% difference in the means would be detectable when single days records were used but with a three day dietary record, a difference of only 5% could be detected. There was no improvement in precision when the period of dietary recording was extended to seven consecutive days. It is, therefore, accepted that a three day dietary diary, which includes a day at the weekend, will provide a valid estimate of energy and macronutrient intake (Bingham et al, 1988). It is difficult to validate dietary diary results



but a relatively simple approach in healthy subjects is to compare daily protein intake with nitrogen output as the latter provides an independent measure of intake (Bingham & Cummings, 1985).

### **Dietary Intake in Liver Disease**

The association of poor dietary intake with under-nutrition among cirrhotic patients has long been recognised (Morgan 1982). For example, Egan and colleagues (1985) observed that the spontaneous dietary intake (hospital diet) of patients with alcoholic liver cirrhosis, was only 50% and 57% of requirements for energy and protein respectively. Conversely, in a study which examined the influences of liver failure and energy expenditure on the response to oral nutrition, Campillo et al (1997) reported an improvement in nutritional status following 30 days of oral nutrition (unenriched hospital diet). However, the methodology used in this study for estimating dietary intake was unclear and appeared to lack formal structure. In this study, reported oral intake "was about" 10.0 MJ (2,400 kcal) per day and without energy dense supplementation would constitute a bulky dietary regimen for these patients.

In marked contrast, Cabre et al (1990) undertook a study of 35 patients with hepatocellular cirrhosis, randomised to receive either enteral tube feeding as the sole source of nutrition or an isocaloric, isonitrogenous oral diet. They found that only in the tube fed group were improvements in clinical and metabolic status observed. The dietary intake of the cirrhotic patients in this study was 5.5 MJ (1,320 kcal) per day which is approximately half that observed by Campillo et al (1997).

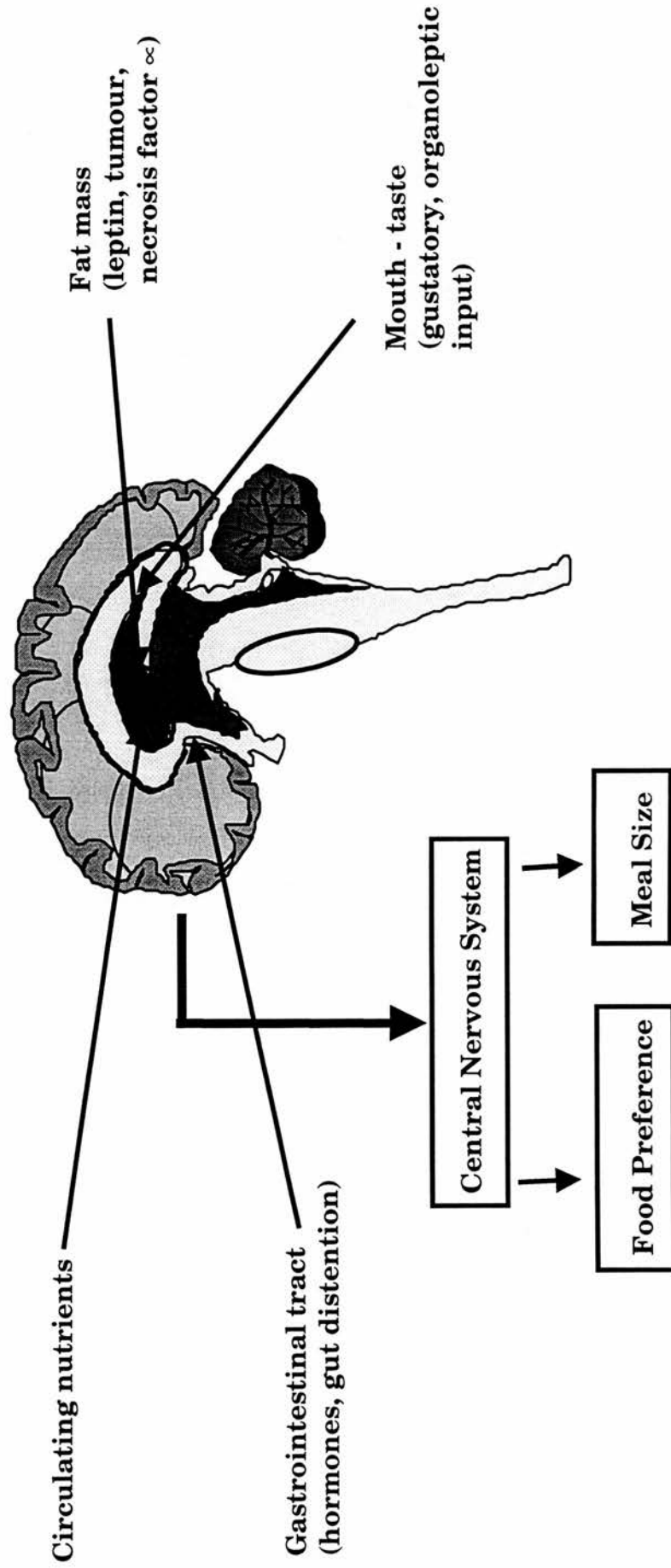
The results from metabolic studies have been used to develop dietary recommendations for patients with liver disease (Plauth et al,

1997). Whilst an adequate nutritional regimen can be readily prescribed, patient compliance is fraught with difficulties and to date no studies have examined spontaneous unrestrained dietary intake in cirrhotic patients. In addition, no prospective longitudinal studies have examined longterm spontaneous oral intake in OLT patients. Given these patients susceptibility to complications such as hypertension, hyperlipidaemia and obesity (Munoz et al, 1991), the consumption of an inappropriate diet may further exacerbate these conditions.

### **Food Preference**

The integration of gastrointestinal derived humoral and neural information occurs in the brain (hypothalamus). This metabolic information is interpreted by the hypothalamus which acts to regulate thermogenesis and determine how much and what sort of food is eaten. The role the brain plays in influencing nutritional intake is outlined in Figure 1.4. Evidence from animal and a limited number of human studies confirm the involvement of a post-ingestive site in food preference. For example, Elizalde and Sclafani (1988), in a study using rats demonstrated a learned and longlasting preference for a non-nutritive flavour when paired with an intra-gastric infusion of glucose. Additionally, the reinforcing effect of a glucose infusion on the consumption of a specific flavour was eliminated when glucose oxidation was inhibited. This highlights the role of post-absorptive influences on ingestive behaviour. Interestingly, further work from this group has shown that rats can also acquire preferences based on the post-ingestive, nutritive properties of fats (Sclafani, 1990).

FIGURE 1.4      PHYSIOLOGICAL INFLUENCES ON NUTRITIONAL INTAKE



Adapted from Wilding, 1997

The higher centres (hypothalamus) integrate signals from the gut and circulation to regulate ingestive behaviour. This metabolic information is used to determine how much and what sort of food is eaten, and the regulation of thermogenesis.

The evidence is unequivocal that hepatic fuel oxidation provides the unconditioned stimulus for controlling nutritional intake (Novin et al, 1985; Tordoff and Friedman, 1986; Tordoff et al, 1990; Friedman, 1997). In a study where glucose was infused into the jugular vein or hepatic portal vein paired with an enteral non-nutritive flavour, Tordoff and Friedman (1986) showed that portal vein catheterised animals demonstrated a preference for the non-nutritive flavour paired with glucose. No flavour preference was exhibited by those animals infused systemically and emphasises the important role of the liver in feeding behaviour. Furthermore, hepatic portal glucose infusions decreased subsequent food intake relative to saline but this effect was not observed in animals infused via the jugular vein.

A later study by Tordoff et al (1990), examined preference for fructose and glucose in animals who were tested after selected hepatic vagotomy or sham vagotomy. This study showed that intact animals preferred flavoured food eaten with a drink of fructose to the flavour eaten with either no sweet drink or glucose. Furthermore, preference for the fructose paired flavour was observed even when fructose and glucose was administered by gavage. However, preference for the fructose paired food depended on an intact hepatic vagus as vagotomised animals exhibited no nutritive preference. This study strongly supports the central role of hepatic signalling on nutrient intake.

The aforementioned studies have focused on animal work and not surprisingly those among humans have centred on food preference in relation to new food product development (Drewnowski and Moskowitz, 1985; Drewnowski, 1995) or in the study of obesity (Mela and Rogers, 1998). There is considerable evidence that the liver controls unconditioned

aspects of food intake (Friedman and Stricker, 1976; Friedman and Sawchenko, 1984; Langhans, 1996) where hepatic and oral afferent neural pathways converge in the hypothalamus and that liver damage alters nutrient intake (Deems and Friedman, 1988a,b; Deems et al, 1993; Madden et al, 1997). Events that influence ingestive behaviour have been considered in isolation and the synergistic effect of metabolic changes that occur in chronic liver disease on food preference and nutritional intake have not been considered. Given the central role of the liver in ingestive behaviour, it seems likely that chronic liver disease would affect the normal function of this metabolic sensor and the information relayed to higher centres could affect food preference which as a consequence would impact on nutritional intake and status. Following OLT the recipient's liver is denervated resulting in a loss of hepatic afferent signalling and could impact on nutritional intake.

## Summary

The metabolic features of chronic liver disease and the prevalence of undernutrition have been widely reported (Crawford et al, 1994; Prijatmoko et al, 1993; Merli et al, 1990; Greco et al, 1998). Although there remains some debate as to the impact of disease severity, primary aetiology and metabolic indices on nutritional status (Gugliemli et al, 1992; Muller et al, 1992; Crawford et al, 1993; Campillo et al, 1997), it has been shown that the presence of undernutrition in liver disease will have a negative effect on clinical outcome (Merli et al, 1996; Selberg et al, 1997). Given this significance of nutritional status on outcome, it adds impetus to identifying strategies that would improve nutritional status. The poor dietary intake of patients with chronic liver disease has been identified as a major problem yet one that may be difficult to resolve (Egan et al, 1985; Neilsen et al, 1993). It is surprising that few studies have examined the relationship of chronic liver disease with factors contributing to poor nutritional status such as anorexia.

It is clear that the liver plays a central role in ingestive behaviour (Tordoff and Friedman, 1986) acting as a sensor for the metabolic control of eating (Friedman, 1995). Under normal conditions hepatic oxidation of lipid and glucose results in changes in hepatocyte membrane potential and modulation of spike frequency in afferent pathways, resulting in satiety (Scharrer et al, 1993). Ultimately it is the convergence in the central nervous system of these neural signals as well as other humoral and cognitive influences that determine the size and duration of an eating episode (Rowlands et al, 1996). Impairment of hepatic neural function and/or alterations in the fuel mix being oxidised may be present in liver disease and could contribute to alterations in ingestive behaviour. It has

also been suggested that impaired functional capacity of the liver may lead to reduced clearance of humoral mediators of satiety such as CCK (Budillon et al, 1996) and this may also contribute to a reduced satiety threshold and result in early meal termination. When considered in the context of patients with chronic liver disease these mechanisms may account for sub optimal energy intakes and contribute to anorexia.

The physiological consequences of progressive liver damage include aberrant metabolism which may cause altered humoral and neural signalling via circulatory and afferent pathways to higher centres. Whether this would affect the metabolic control of ingestive behaviour or development of food preferences is unclear. Conversely, patients who undergo OLT have a propensity towards obesity (Palmer et al, 1991). Understanding the mechanisms involved in this OLT weight gain is important where complications such as diabetes, hypertension and hyperlipidaemia may be exacerbated in overweight or obese OLT patients. Following OLT the recipient's liver is denervated and results in the total loss of extrinsic innervation with no signalling either from or to higher centres and it could be postulated that this would have a significant influence on ingestive behaviour.

It is becoming clear that understanding the influence that altered metabolism may have on nutritional intake may have important implications for nutritional management before and after OLT. This thesis sought to examine factors which may be associated with anorexia in chronic liver disease and weight gain following OLT.

## Specific Aims of the Thesis

The main aims of this thesis were to examine:

- (i) the influence of energy and substrate metabolism on dietary intake, macronutrient preference and nutritional status in chronic liver disease and following orthotopic liver transplantation.
- (ii) the contribution of factors such as immunosuppressive therapy, energy expenditure and energy intake on post-transplant weight gain.

To study this, the specific aims were to:

- (a) determine nutritional status in cirrhotic patients and in a sub group of patients following OLT.
- (b) measure substrate oxidation rates in the fasted and fed state among stable cirrhotic patients and in a sub group of patients following OLT.
- (c) measure the glucose and insulin response to a standardised test meal in stable cirrhotic patients and in a sub group of patients following OLT.



- (d) estimate the spontaneous macronutrient intake in stable cirrhotic patients and in a sub group of patients following OLT.
- (e) assess macronutrient preference in stable cirrhotic patients and in a sub group of patients following OLT.
- (f) determine the relationship between nutrient handling, nutritional intake/preference and nutritional status in stable cirrhotic patients and in a sub group of patients following OLT.
- (g) determine the association between OLT weight change and immunosuppressive drug regimens.

## CHAPTER 2

### METHODS

#### Subjects

Patients who were referred to the Scottish Liver Transplant Unit under consideration for orthotopic liver transplantation between December 1995 and August 1997 were eligible for study. All patients had histologically proven liver cirrhosis and those with alcohol related disease had been abstinent for a minimum period of six months. Patients with known diabetes, renal disease, lung disease, thyroid dysfunction, active inflammatory bowel disease, sepsis, severe ascites and grade III/IV encephalopathy were excluded from the study. No patient had evidence of neoplastic disease before or at surgery. A group of healthy subjects was recruited from hospital personnel, friends and relatives of the investigator. No healthy subject had a chronic illness or was on any medication.

Patients were studied at assessment for transplantation and those who subsequently underwent liver transplantation were studied at three, six and nine months following discharge from hospital. All transplant recipients underwent a conventional OLT and in no patient was an auxillary graft undertaken which left part or all of the native liver. All procedures relating to the study were carefully explained to subjects who were required to give written consent. The study protocol was reviewed and approved by Lothian Health Board Medical/Clinical Oncology Research Ethics Sub-Committee.

## Indirect Calorimetry Studies

### Measurement of Energy Expenditure

Energy expenditure was measured using a mobile indirect calorimeter (Deltatrac Datex Monitor, Engstrom, Kent, United Kingdom). The Deltatrac is a ventilated (open hood) calorimeter and measurements during spontaneous breathing are made by sucking air through a canopy at a known constant rate (approximately 40 litres/minute). The difference between the inspired and expired oxygen fractions are measured with a para-magnetic oxygen sensor and the expired CO<sub>2</sub> fraction is measured with an infra-red CO<sub>2</sub> sensor. Flow rate through the canopy is in the first instance determined by the manufacturer and this value is set in the micro-processor software. Calculation by the micro-processor of VO<sub>2</sub>, VCO<sub>2</sub>, RQ and energy expenditure occurs several times a minute. The Weir (1949) formula was used to determine energy expenditure:-

$$\text{energy expenditure (kcal)} = 3.941 \text{ VO}_2 + 1.106 \text{ VCO}_2 - 2.17 \text{ N}$$

$$\text{kJ} = \text{kcal} \times 4.12$$

(where gases are expressed in litres and nitrogen (N) in grams and 4.12 kcal/kJ)

### Calibration

To check the performance of the calorimeter, the manufacturers alcohol burning kit and protocol was used to check flow rate and RQ, these checks were carried out on a monthly basis. New flow constant was determined using the formula:-

$$\text{new flow} = 1.03 \times \frac{3820 \text{ ml}}{\text{VCO}_2 \text{ ml}} \times \text{old flow}$$

(where a 5 ml dose of 100% ethanol will produce 3820 ml CO<sub>2</sub>)

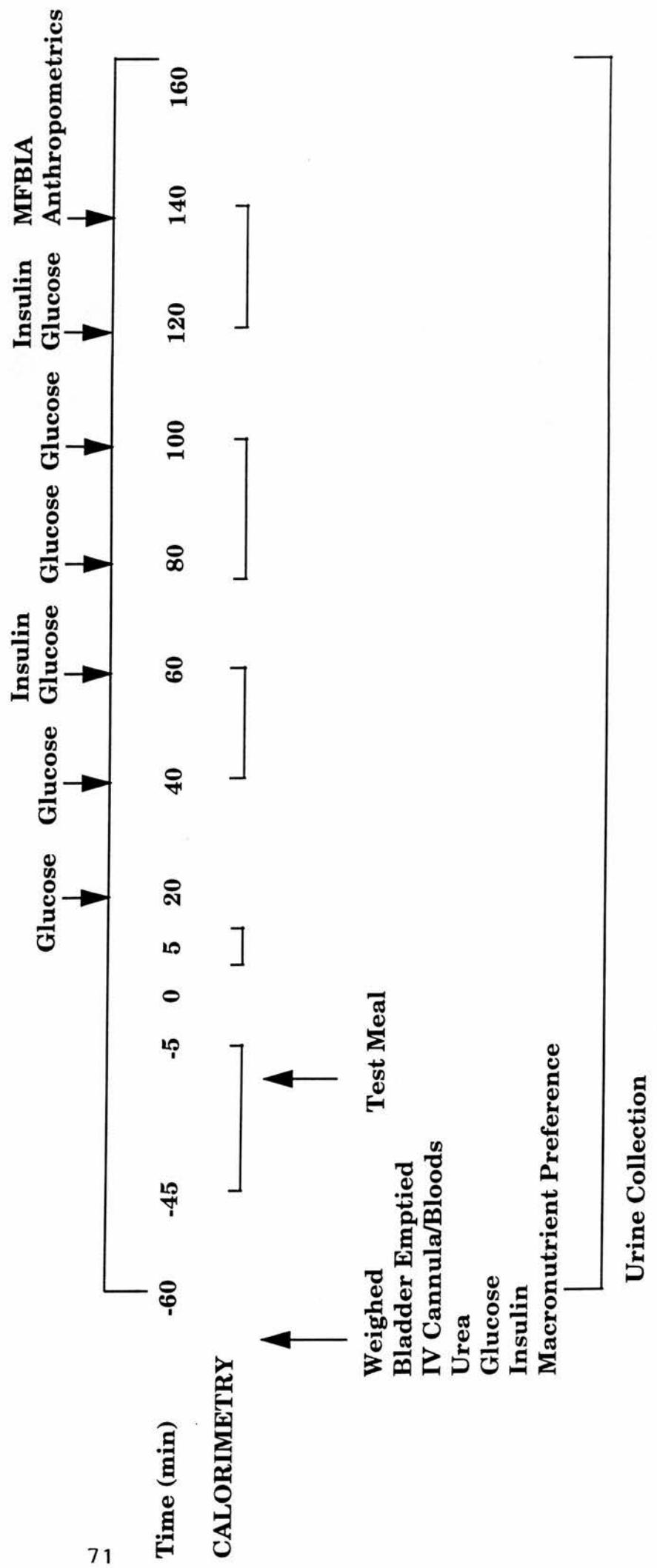
Throughout the study period, the CoV was <3%. Respiratory quotient validation involved a 30 minute period of burning pure ethanol (RQ = 0.67) in a lamp with wick and in a glass container to simulate the canopy mode. In addition to routine performance checks carried out by the investigator, the calorimeter was also fully serviced twice yearly by an engineer from the manufacturer.

### Calorimetry Measurements

Prior to each period of measurement, the calorimeter was switched on and the monitor dimmed at least 12 hours before use to stabilise analysers. To ensure accurate gas exchange measurements, calibration of gas sensors using carbogen (5% CO<sub>2</sub>, 95% O<sub>2</sub>; Quick CAL, calibration gas, Datex, Helsinki, Finland) were performed at the beginning and end of each measurement period. Daily calibration for atmospheric pressure using a barometer (Munro Ltd., London, UK) was carried out to avoid inaccurate pressure calibration which could result in an error in flow constant. All calorimetry measurements were performed on steady state subjects in a quiet area of the ward in thermally comfortable conditions. Subjects were fully briefed about the calorimeter's basic operation and to ensure volunteers remained awake, the researcher (RAR) remained with the subject during measurement periods. Measurements of energy expenditure in the resting and fed state were performed on five occasions over three hours (Figure 2.1).

**FIGURE 2.1**

**SCHEMATIC OUTLINE OF STUDY PROTOCOL**



### Predicted Energy Expenditure

Predicted energy expenditure was derived from the age specific Schofield (1985) equation. An example of the equation applied to subjects aged between 30-60 years of age is as follows:

$$\text{males} \quad \text{REE} = (11.4 \times \text{weight}) + 870$$

$$\text{females} \quad \text{REE} = (8.1 \times \text{weight}) + 842$$

Predicted results were expressed as a percentage of measured energy expenditure.

### Respiratory Quotient

The ratio of  $\text{VCO}_2$  and  $\text{VO}_2$  is a useful indicator of the type of substrate oxidation where the normal physiological range is 0.7 - 1.0 with an RQ of 0.7 representing net lipid oxidation and an RQ of 1.0 indicating net CHO oxidation. In this study, measurement of urinary nitrogen excretion was performed to permit calculation of NPRQ and substrate oxidation rates. By subtracting the volume of oxygen consumed and carbon dioxide produced for every gram of protein oxidised from total  $\text{VO}_2$  and  $\text{VCO}_2$ , then non-protein  $\text{VO}_2$  and  $\text{VCO}_2$  may be derived. The following equation of Jequier et al (1987) were used to determine NPRQ:-

$$\text{PVCO}_2 = \text{N} \times 6.25 \times 0.774$$

$$\text{PVO}_2 = \text{N} \times 6.25 \times 0.966$$

$$\text{NPRQ} = \frac{\text{VCO}_2 - \text{PVCO}_2}{\text{VO}_2 - \text{PVO}_2}$$

where:-

$\text{PVCO}_2$  = carbon dioxide production rate due to protein metabolism (litres/min)

$PVO_2$  = oxygen consumption rate due to protein metabolism  
(litres/min)

N = urinary nitrogen excretion (g/day)

The above formula was used as it is valid in the presence of lipogenesis (Ben-Porat et al, 1983) or ketogenesis (Frayn, 1983).

#### Calculation of Substrate Oxidation Rates

Normally, measurement of respiratory gas exchange is indicative of all the oxidative processes occurring in the body. By measuring a subject's  $VO_2$  consumption,  $VCO_2$  production and urinary nitrogen excretion, substrate oxidation rates may be derived.

An index of protein oxidation over the test was obtained from urinary nitrogen excretion period (6 hour collection). To correct for changes in the urea pool during the period of study, blood urea concentrations were measured at baseline and at the end of the study period. The correction factor described by Jequier et al (1987) was only used when the change in blood urea concentration exceeded 5%.

$$\text{urea correction} = \frac{\text{change urea nitrogen (g)} \times \text{urea pool}}{4 \times 1000}$$

The oxidation rates for fat, carbohydrate and protein as calculated using the following stoichiometric equations (Jequier et al, 1987):-

$$\text{carbohydrate oxidation (g/min)} = NPVO_2 \times \frac{(NPRO - 0.696)}{0.304 \times 0.746}$$

$$\text{fat oxidation (g/min)} = NPVO_2 \times \frac{(1 - NPRO)}{0.304 \times 2.012}$$

$$\text{protein oxidation (g/min)} = \frac{PVO_2}{0.966}$$

These formulae are similar to others used in studies of substrate oxidation (Frayn et al, 1983; Elia & Livesey, 1988) and unlike the tables of Elia and Livesey (1988) or the more complex formulae of Frayn (1983) the stoichiometric equations of Jequier et al (1987) were easily incorporated into the database programme used in the current study (Filemaker Pro, Claris Corp., USA).

## **Anthropometry**

### Height and Weight

Subject's height were measured without shoes using a stadiometer (Docherty Medical, London) to the nearest centimeter (0.01 m). Subjects were weighed in light clothing to an accuracy of 100 gms (0.01 kg) on mobile sitting scales (Weymed Ltd., United Kingdom) and body mass index ( $\text{weight} \div \text{height}^2$ ) was calculated and used as a marker of leanness or obesity where a desirable BMI for men and women is between 20-25 (Roche et al, 1981). On entry to the study, patients were asked to subjectively report any weight loss during the preceding year. This was expressed as a percentage of actual body weight and reported as recent weight loss.

### Arm Anthropometry

As a substantial proportion of the fat mass lies subcutaneously, skinfold measurements provide a method of quantifying the amount and in longitudinal studies determines changes in this energy reserve. Although many sites may be used in assessing fat reserves, only TSF measurements which correlate strongly with reference techniques of measuring TSF (Booth, 1966) were used in this study. The upper arm is



less affected by bed rest or fluid retention (Wicks et al, 1995). In addition, it has the added advantage of being easily accessed and the measurement itself is minimally intrusive. This was particularly important in the current investigation where the subjects continued support was paramount.

Measurement of TSF was performed using Harpenden Skinfold Calipers (Holtain Ltd., Bryberian, Crymmych, Pembrokeshire, United Kingdom) by the author (RAR). Triceps skinfold thickness was measured by marking the mid-point between the acromion and olecranon processes in the dependent non-dominant arm with the elbow joint flexed to 90°. A skinfold 1 cm above this point overlying the triceps muscle was pinched between first finger and thumb and three readings of the triceps skinfold thickness was obtained. Readings were taken three times and the average taken as an absolute value.

In order to make comparisons between males and females, TSF values were expressed as a percentage of standard where males = 12.5 mm and females = 16.5 mm (Jelliffe 1966). In the current study, these standards were considered appropriate as all subjects were of a similar age and the Jelliffe (1966) standards are comparable with the 50th percentile values from other population studies (Frisancho 1974; Bishop et al, 1981).

In estimating AMC, indicative of skeletal muscle mass, a number of assumptions are made: firstly, the upper arm is considered a perfect circle and no allowance is made for the bone in the arm. Secondly, this quantitative assessment of skeletal muscle mass is predictive of LBM. Mid upper arm circumference was measured, at the mid point of the upper arm, using a flexible measuring tape. From

measurement of TSF and mid upper arm circumference, AMC is derived using the formula:-

$$AMC_{(cm)} = MUAC_{(cm)} - (3.14 \times TSF_{(mm)})$$

in which AMC, mid upper arm circumference and TSF are expressed in centimetres. To allow gender comparisons, results were expressed as a percentage of standard, where mean values for AMC were 25.3 cm and 23.2 cm for males and females respectively (Jelliffe, 1966). These figures are comparable with other population studies (Frisancho 1974; Bishop et al, 1981).

Arm anthropometry was performed on subjects at the end of every study period.

#### **Multi-Frequency Bioelectrical Impedance Analysis**

This was performed on the day of investigation using a Xitron 4000B Analyser (Xitron Technologies, San Diego, California, USA) operated at 200  $\mu$ A at the previously defined optimal frequencies of 5 and 200 KHz (Hannan et al 1994). The alternating current was passed between a set of pre-gelled current injection electrodes (Bodystat, Isle of Man, United Kingdom). These were placed on the right hand and foot, just proximal to the third metacarpal and metatarsal bones respectively. Similar detection electrodes were placed on the right wrist between the radius and ulnar and on the right ankle between the malleoli. Measurements were taken on the morning of study with the subject in the supine position, with legs and leads apart. Total body water and extracellular water was calculated from predictive equations previously derived from isotopic studies on surgical patients

performed in our own unit (Hannan et al, 1995). These equations are as follows:-

$$ECW_{(L)} = 0.1782 \frac{(Ht^2)}{(R_5)} + 0.0688 \text{ wt} + 3.771$$

$$TBW_{(L)} = 0.2391 \frac{(Ht^2)}{(R_{200})} + 0.1889 \text{ wt} + 2.971 \text{ Sex (female = 0, male = 1)} + 5.4641$$

where: ECW = extra cellular water; TBW = total body water;  
Ht = height; wt = weight;  $R_5$  = resistance measurement at 5 Khz;  
 $R_{200}$  = resistance measurement at 200 Khz

By subtracting extracellular water from total body water, an estimate of intracellular water and body cell mass was derived (Shizgal 1983) as follows:

$$ICW = TBW - ECW$$

$$BCM = \frac{ICW}{0.70}$$

where: ICW = intracellular water; BCM = body cell mass

In this study, analysis of body composition by total body potassium measurements or isotopic studies were not possible. Bioelectrical impedance among patients with mild and moderate ascites has been validated with total body potassium measurements as a method of assessing the metabolically active component of body composition, the BCM in populations of cirrhotics (Muller et al, 1992).

## Biochemical Measurements

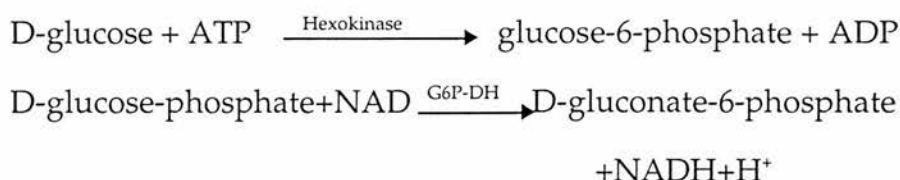
### Blood Samples

At least 40 minutes before investigation, an indwelling cannula (Venflon 2, BOC Ohmeda AB, Helsingborg, Sweden) was sited in the brachial vein and kept patent with a minimal infusion of normal saline.

Blood samples were taken at the time points indicated in the schema shown in Figure 2.1. Blood samples (lithium heparin x 2, glucose x 7) were stored in an ice filled flask and serum Gel samples (x 3) were kept at room temperature. Once investigations were complete, samples were centrifuged without delay at 2000 g at 2 - 4°C for ten minutes. Serum and plasma samples were stored at -40°C and analysed in batches for plasma insulin, serum glucose and blood urea (Clinical Chemistry, Western General Hospital, Edinburgh).

### Plasma glucose

Glucose concentration was measured by the timed end-point method on the Roche Cobas Mira Plus (Roche Diagnostics Ltd, Lewes, Sussex, UK) analyser.



ADP = adenine di-phosphate

G6P-DH = glucose-6-phosphate-dehydrogenase

NAD = nicotinamide adenine di-nucleotide

NADH= nicotinamide adenine di-nucleotide (reduced form)

The formation NADH is directly related to the glucose concentration and is measured photometrically at 340 nm. The inter assay coefficient of variation is less than 1.5%.

### Plasma insulin

Insulin was measured by a two site immunoenzymometric assay on a the AIA-600 random access automated enzyme immunoassay system (Tosoh Corporation, Tokyo, Japan). During an incubation step, insulin in the sample is sandwiched between an antibody immobilised on magnetic solid phase and an enzyme labelled monoclonal antibody (alkaline phosphatase). The magnetic beads are subsequently washed to remove unbound enzyme labelled antibody and incubated with 4- methylumbelliferyl phosphate which is a fluorogenic substrate to alkaline phosphatase. The rate of fluorescence produced is directly proportional to the concentration of insulin in the sample. The sensitivity of the assay is 2.0 mU/L and the inter-assay coefficient of variation is less than 5% at all levels.

### C-Reactive protein (CRP)

C-Reactive protein was measured by an ELISA (enzyme link immuno-sorbent assay) procedure using Rabbit Anti-Human CRP as the detection antibody (Dako, Glostrup, Denmark). After coating wells with diluted antibody (1 : 5,000), microtitre plates were covered and incubated overnight. Plates were then blocked with bovine serum albumin (1%) and washed. Patient samples were diluted (1 : 10,000) in phosphate buffer and saline and added to each well. In addition, CRP standards (0.5 µg/l - 50 µg/l; Human Serum CRP Calibrator, Deko, Glostrup, Denmark) (TBS Buffer) were analysed. Duplicates of samples, standards and blanks were placed in wells using previously designed plate maps and incubated overnight.

The plates were washed and the detection antibody (Peroxidase-Conjugated Rabbit Anti-Human CRP, Dako, Glostrup, Denmark) was added to each well, incubated and the plates washed. The chromogenic substrate 5,5<sup>1</sup>,3,3<sup>1</sup> tetra methyl benzidine (Dako Patts, Dako, Glostrup, Denmark) was added to allow colour development, plates were covered to protect them from light and incubated for 15 minutes. Colour development was stopped by adding 20 µl of 1 M sulphuric acid to each well and plates were read within one hour using an automated plate reader (Dynatech, West Sussex, UK). The CRP concentration was calculated in each patient by reading values from the standard curve using Absay gap computer software (Biosoft, Cambridge, United Kingdom). The lower limit of sensitivity taking into account sample dilutions was 150 µg/l<sup>1</sup>. The intra-assay coefficient of variation was 7% and inter-assay coefficient of variation was 15% at a concentration of 1 : 10,000.

#### Urinary nitrogen

Prior to investigation, subjects were asked to empty their bladders. Urine was collected over the 6 hour test period and also over the subsequent 24 hour period in graduated plastic storage bottles (2500 ml). The 6 hour urine collection was only taken in those subjects entered into the test meal limb of the study. This permitted protein oxidation to be calculated over the study period.

Urine samples were batched, frozen and subsequently analysed using a nitrogen analyser (LECO-FP328, St. Joseph's, Michigan, USA). A 200 µl urine sample was placed in a smooth wall tin capsule (LECO, Michigan, USA) and underwent the analysis cycle. The encapsulated

sample of urine was purged of any atmospheric gases, dropped into a hot furnace (850°C) and then flushed with pure oxygen for rapid combustion. The products of combustion CO<sub>2</sub>, H<sub>2</sub>O, NO and N<sub>2</sub> were passed through the furnace filter and then the thermoelectric cooler to remove most of the water. A sample aliquot of the gases is then swept through hot copper to remove oxygen and change NO to N<sub>2</sub>, then through Lecosorb (LECO, Michigan, USA) and Anhydrone (LECO, Michigan, USA) to remove CO<sub>2</sub> and H<sub>2</sub>O respectively. The remaining nitrogen is measured by the thermal conductivity cell.

Results are given as weight percentage of nitrogen and using the subjects total urine volume, urinary nitrogen may be calculated.

### Test Meal

On the day of investigation, the test meal was prepared as follows:-

#### Test Meal

20 g Polycal (Nutricia, Trowbridge, UK)  
200 ml Fortisip (Nutricia, Trowbridge, UK)  
50 ml water

#### Nutritional Content (volume 250 ml)

Energy	1596 kJ (380 kcal)
Carbohydrate	56 g (59% CHO)
Fat	13 g (31% fat)
Protein	10 g (10% protein)

Ingredients for the test meal and subjects prescription (15 kJ/kg) were weighed on scales accurate to 0.1 g (Mettler, Instrumente, Zurich, Switzerland). Following the protocol outlined in Figure 2.1, subjects were required to consume the test meal over five minutes.

## **Nutrient Intake**

In order to estimate subjects' dietary intake, food diaries (recording intake for two week days and one day at the weekend) were kept by subjects for three days in the week following hospital discharge. Clear verbal and written instructions were given to all subjects on how to complete diet diaries. Additionally, in order to test understanding of diary completion, patients were questioned after the diet diary interview and where appropriate, further instruction was given. Subjects who did not return their diaries within ten days were given one telephone reminder.

Diaries were transposed to a standard data sheet by the investigator and where required the Food Portion Sizes (MAFF, 1993) was used to determine portion sizes. Data was analysed using a dietary computer analysis programme (Comp-eat, Nutrition Systems, London).

## **Macronutrient Preference**

Using the methodology of Drewnowski and Greenwood (1983) preference for fat and carbohydrate was determined using four commercially produced ice creams (a real food combining fat and carbohydrate) as the test stimuli to determine macronutrient preference.

Each ice cream varied in macronutrient content (Table 2.1) but all were vanilla in flavour. The composition of the ice creams used did not change over the study period. Approximately 15 ml of ice cream was served in a plastic beaker which was numerically coded (Table 2.1). Subjects randomly selected samples for testing and in



TABLE 2.1                      MACRONUTRIENT VALUES OF ICE CREAMS (per 100 ml)

Sample Code	Wt./100 ml	Protein (g)	Fat (g)	CHO (g)	Energy (KJ), (kcal)	Type
A	166	6.5	19.0	29.7	1339 (321)	High fat, High CHO
B	114	4.5	18.4	20.0	1078 (258)	High fat, Mod. CHO
C	181	3.8	0.9	27.8	564 (135)	Low fat, High CHO
D	217	2.8	4.8	20.8	589 (141)	Low fat, Mod. CHO

A = Mackie's Traditional; Westertown, Aberdeen, UK.  
B = Haagan Dazs, Middlesex, UK.  
C = Walls Too Good To Be True, Birds Eye Walls Ltd., Surrey, UK.  
D = Heinz Weight Watchers, HJ Heinz Co. Ltd., Middlesex, UK

order to mask any visual differences, eye shades were worn during the tasting procedure. Hedonic responses (degree of liking) were recorded for each coded sample on a nine point hedonic scale (Peryam and Pilgrim, 1955) anchored by the responses “dislike extremely” and “like extremely” as shown below:

- |    |   |                         |
|----|---|-------------------------|
| 1. | = | Dislike extremely       |
| 2  | = | Dislike very much       |
| 3  | = | Dislike moderately      |
| 4  | = | Dislike slightly        |
| 5  | = | Neither like or dislike |
| 6  | = | Like slightly           |
| 7  | = | Like moderately         |
| 8  | = | Like very much          |
| 9  | = | Like extremely          |

On the morning of study subjects tasted and swallowed samples and were subsequently asked to elicit pleasantness ratings. Re-tasting was permitted. Palate cleansing using water (Evian, France) was performed before each tasting session and following ingestion of each sample. Macronutrient preference was re-examined in all subjects and no significant differences between the first and second tasting were observed (Wilcoxon-Signed Rank test).

### Statistics

All statistics were performed on an Apple Macintosh Centris (616) computer using a commercially available statistics package StatView 4.02 (Abacus Concepts Inc., California, USA). Where several groups of data were compared, analysis of variance (ANOVA) was

used to test whether there were significant differences between groups. Longitudinal changes were analysed using repeated measures ANOVA and the post hoc Scheffe's test. The effects of the test meal in cirrhotic patients was analysed by two factor repeated measures ANOVA and the post-hoc Scheffe's test was used in comparing means.

In pairwise comparisons, Student's *t*-tests was used for normally distributed data and the non-parametric Mann-Whitney *U* test and Wilcoxon Signed Rank test was used for pairwise comparisons if data was not normally distributed. Following OLT, analysis of test meal data was performed using Friedman's two way analysis of variance and Wilcoxon Signed Rank test was used to detect differences.

Food preference results were expressed as median and inter-quartile range (IQR) and values outwith the 25th percentile and 75th percentile were represented in the figures individually. In analysis of food preference data, differences between groups were determined using Kruskal-Wallis one way analysis of variance and Mann-Whitney *U* test. Differences within groups were determined using Friedman's two-way analysis of variance and Wilcoxon Signed Rank test.

For descriptive purposes, data has been expressed as mean ( $\pm$  SEM) to facilitate comparison with other published results. Pearson's correlation coefficient was used to determine associations between variables. Multiple stepwise regression was performed using fat mass as the dependent variable and cumulative drug dose, energy expenditure and energy intake as the independent variables.

Differences were considered significant when the probability of their arising by random sampling error was less than 1 in 20 ( $p < 0.05$ ).

## CHAPTER 3

### THE CLINICAL AND NUTRITIONAL STATUS IN LIVER CIRRHOSIS AND LIVER TRANSPLANT RECIPIENTS

The poor nutritional status of hospitalised patients with chronic liver disease has been widely reported (Crawford et al, 1994; Wicks et al, 1995; Caregaro et al, 1996). However, following OLT sustained and rapid weight gain has been reported (Munoz et al, 1991). This increase in body mass could be attributed to a return to pre-illness weight. However, given the duration of continued weight gain (up to 16 months) and that patients who were overweight prior to transplantation (40%) became more overweight following surgery, suggests an increase beyond that of 'usual' body weight (Palmer et al, 1991).

The current study considered the nutritional and metabolic status of cirrhotic patients not admitted for symptomatic control of acute exacerbations of their liver disease. This permitted the study of a population with a chronic rather than an acute on-chronic disease process. In addition, a sub-group of these cirrhotic patients were listed for and subsequently underwent OLT. These patients were studied at three monthly intervals up to one year following liver transplantation to allow longitudinal study of changes in patients nutritional status.

#### Patient and Methods

Patients admitted for consideration of orthotopic liver transplantation, a sub-group of cirrhotic patients who underwent OLT and a group of 18 healthy volunteers were recruited. Studies were performed in the Scottish Liver Transplant Unit between January 1996

and July 1998. Sixty-seven cirrhotic patients with histologically proven cirrhosis were recruited. Thirty-five patients had cholestatic disease (34 primary biliary cirrhosis, one primary sclerosing cholangitis) and 32 hepatocellular cirrhosis (23 alcoholic cirrhosis, three cryptogenic cirrhosis and six Hepatitis C). Thirty-three patients from this cohort were transplanted. Those cirrhotic patients submitted to OLT were reviewed on a three monthly basis, on at least three occasions after discharge from hospital. Four patients died in the early postoperative period and six patients were subsequently excluded because of the presence of significant complications (prolonged sepsis  $n = 4$ ; bile duct stricture  $n = 1$ ; hepatic vein stenosis  $n = 1$ ). Therefore, 23 OLT patients were nutritionally assessed following discharge at three monthly intervals on at least three occasions.

## **Methods**

Body composition was determined from anthropometric and MFBLA measurements. Resting energy expenditure was measured using indirect calorimetry. Serum albumin concentration and C reactive protein were used as biochemical markers of disease severity and inflammatory response respectively. A more detailed account of the methodology is given in Chapter 2.

## **Results**

### **Cirrhotic Patients**

In the cirrhotic patient group, pharmacological regimens during the assessment period included diuretic therapy (41 patients), vitamin supplements (13 patients) and lactulose (12 patients). Comparison of nutritional, clinical and metabolic status of cirrhotic patients are shown in Tables 3.1 and 3.2.

TABLE 3.1 NUTRITIONAL AND CLINICAL STATUS OF CIRRHOTIC PATIENTS AND CONTROLS

	Cirrhotic Patients	Controls
n	67	18
Male : Female	26 : 41	9 : 9
Age	54.5 (1.1)	50.2 (2.7)
Weight (kg)	66.7 (1.7) <sup>b</sup>	76.9 (2.9)
Ideal body weight (%)	107 (2.8)	117 (3.5)
Recent weight loss (%)	-7 (1.2) <sup>b</sup>	0
Body mass index	24.1 (0.6)	26.4 (0.9)
Triceps skinfold thickness (mm)	12.4 (0.7) <sup>a</sup>	15.9 (1.7)
(%)	82 (4.2) <sup>b</sup>	108 (9.2)
Arm muscle circumference (cm)	22.4 (0.4) <sup>c</sup>	25.6 (0.7)
(%)	94 (1.6) <sup>b</sup>	102 (3.3)
Serum albumin concentration (g/l)	30.7 (0.6)	35 - 45 (reference range)
C-Reactive protein (mg/l)	10.2 (2.1)	9.4 (5.1)
Child's Pugh score	8.7 (0.3)	-
Child's Pugh score A n = 14		
Child's Pugh score B n = 26		
Child's Pugh score C n = 27		

Mean (± SEM)

a = p<0.05 versus controls

b = p<0.01 versus controls

c = p<0.001 versus controls

**TABLE 3.2 METABOLIC STATUS OF CIRRHOTIC PATIENTS  
AND CONTROLS**

	<b>Cirrhotic Patients</b>	<b>Controls</b>
Resting energy expenditure (kcal/kg)	20.6 (0.4)	19.1 (0.5)
(kcal/kg BCM)	59.8 (1.5)	55.8 (1.3)
Predicted energy expenditure (%)	91 (1.9)	94 (2.9)
Non protein respiratory quotient	0.81 (0.01) <sup>a</sup>	0.84 (0.01)
Urinary nitrogen excretion (g/day)	6.2 (0.5) <sup>a</sup>	8.6 (0.9)

Mean ( $\pm$  SEM)

a =  $p < 0.05$  versus controls

Cirrhotic patients had lower body weight than controls ( $p<0.01$ ) but when weight comparisons between these groups were normalised for gender using percentage ideal body weight (Statistical Bulletin, 1983) no differences between the cirrhotic patients and control subjects were apparent. Cirrhotic patients had a reported mean recent weight loss of 7%. Triceps skinfold thickness and AMC were significantly lower among cirrhotic patients when compared with controls (TSF =  $p<0.05$ ; AMC =  $p<0.001$ ). Arm anthropometry results were normalised for gender and expressed as a percentage of standard. Both TSF and AMC were lower in cirrhotic patients when compared with controls (TSF =  $p<0.01$ ; AMC =  $p<0.01$ ). Respiratory quotient and urinary nitrogen excretion were significantly lower in patients when compared with controls ( $p<0.05$ ).

Table 3.3 shows MFBIA results in male and female cirrhotic patients. Control subjects and percentage values were used to facilitate gender comparison. Body cell mass was significantly lower in female cirrhotic patients when compared with female controls ( $p<0.05$ ) but when expressed as a percentage of body weight, there were no significant differences between patient groups and controls. Fat mass was significantly lower in female cirrhotic patients when compared with female controls ( $p<0.05$ ). Additionally, when expressed as a percentage of body weight, fat mass was significantly lower in both male and female cirrhotic patients when compared to their healthy counterparts ( $p<0.05$ ).

In the cirrhotic population, there was a highly significant relationship between measured energy expenditure and the predicted energy expenditure estimated by the Schofield (1985) equation (Figure 3.1). In this study, energy expenditure was measured by indirect calorimetry and given that BCM is the oxygen consuming



TABLE 3.3 COMPARISONS BETWEEN MFBIA RESULTS FOR MALE CIRRHOTIC PATIENTS AND CONTROLS AND FEMALE CIRRHOTIC PATIENTS AND CONTROLS

	Cirrhotics		Controls	
	Male (n = 26)	Female (n = 41)	Male (n = 9)	Female (n = 9)
Body weight (kg)	74.1 (2.2)	62.1 (2.1)	83.2 (3.8)	70.9 (3.3)
% Ideal body weight	108 (3.2)	107 (4.2)	113 (4.9)	119 (5.0)
Total body water (kg) (%)	38.8 (1.1) 51 (0.8)	29.4 (0.8) 48 (1.0)	41.6 (1.6) 50 (0.9)	31.4 (0.8) 44 (1.1)
Lean body mass (kg) (%)	53.1 (1.5) 67 (1.5)	40.2 (1.1) 66 (1.4)	57.1 (2.3) 68 (1.2)	43.1 (1.1) 61 (1.5)
Body cell mass (kg) (%)	28.5 (0.8) 37 (1.0)	19.7 <sup>b</sup> (0.7) 32 (1.0)	31.2 (1.5) 38 (1.0)	22.5 (0.7) 33 (0.7)
Fat mass (kg) (%)	23.9 (1.4) 30 <sup>a</sup> (1.0)	21.2 <sup>b</sup> (1.6) 31 <sup>b</sup> (1.3)	26.0 (1.9) 34 (1.2)	27.9 (2.2) 37 (1.7)

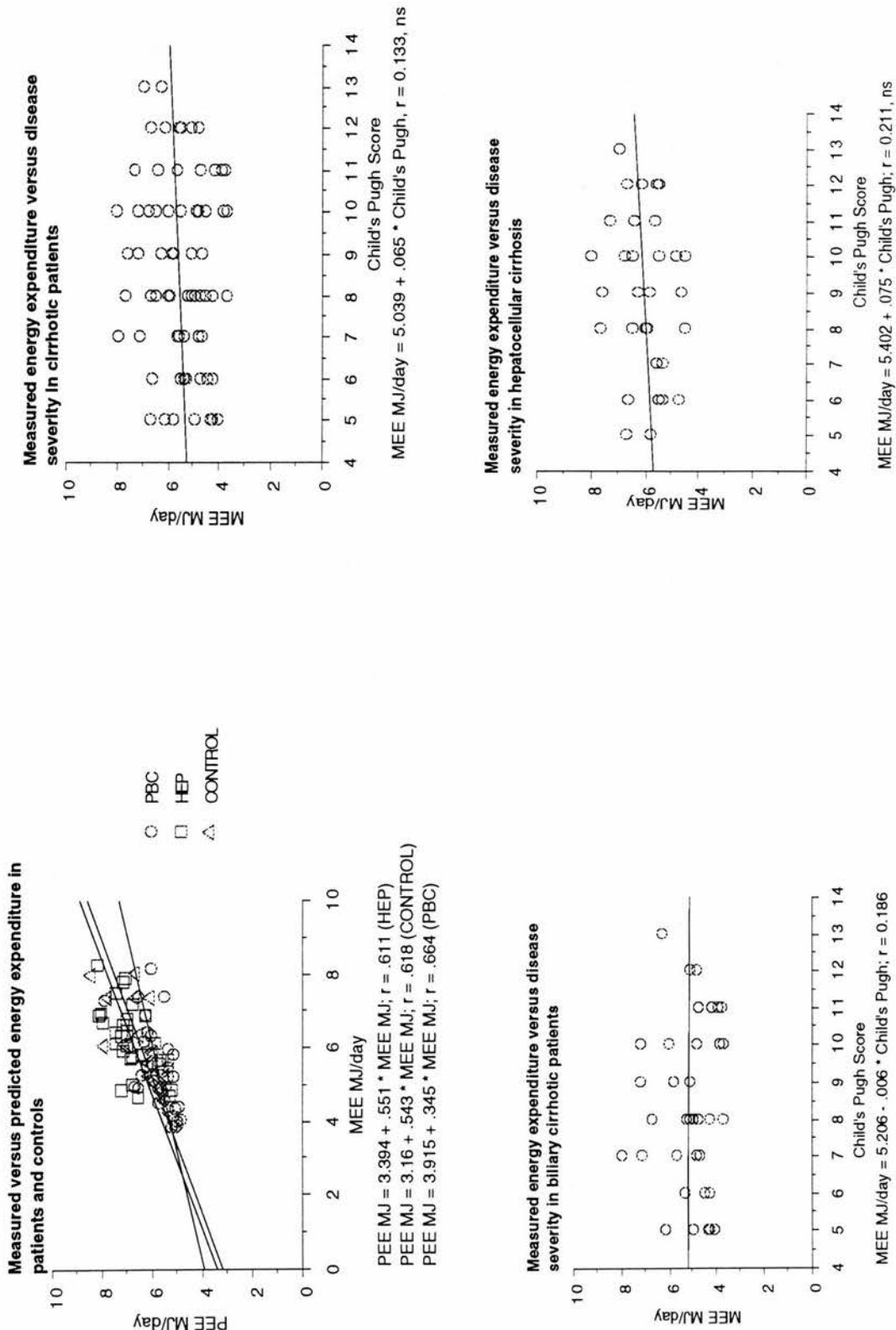
Mean ( $\pm$  SEM)

a =  $p < 0.05$  male cirrhotic patients versus male controls

b =  $p < 0.05$  female cirrhotic patients versus female controls

Equation used to calculate BCM is given in Chapter 2, page 77.

**FIGURE 3.1 MEASURED ENERGY EXPENDITURE VERSUS PREDICTED, PRIMARY PATHOLOGY AND DISEASE SEVERITY IN CIRRHOTIC PATIENTS**



component of body composition, the relationship between measured energy expenditure and BCM was examined in both patients and controls (Figure 3.2). Regression analysis showed a strong positive relationship between measured energy expenditure and BCM in cirrhotic patients and controls ( $r = 0.765$ ;  $p < 0.0001$ : controls  $r = 0.957$ ;  $p < 0.0001$ ). This relationship held in patients with and without ascites (ascites,  $r = 0.809$ ;  $p < 0.0001$ : no ascites,  $r = 0.732$ ;  $p < 0.0001$ ). This finding supports the use of MFBIA in determining body composition in populations of cirrhotic patients with mild and moderate ascites. No patient in the present study had severe ascites. In order to facilitate comparisons between cirrhotic patients and controls, MFBIA is used in the expression of energy expenditure data as per kilogram body cell mass.

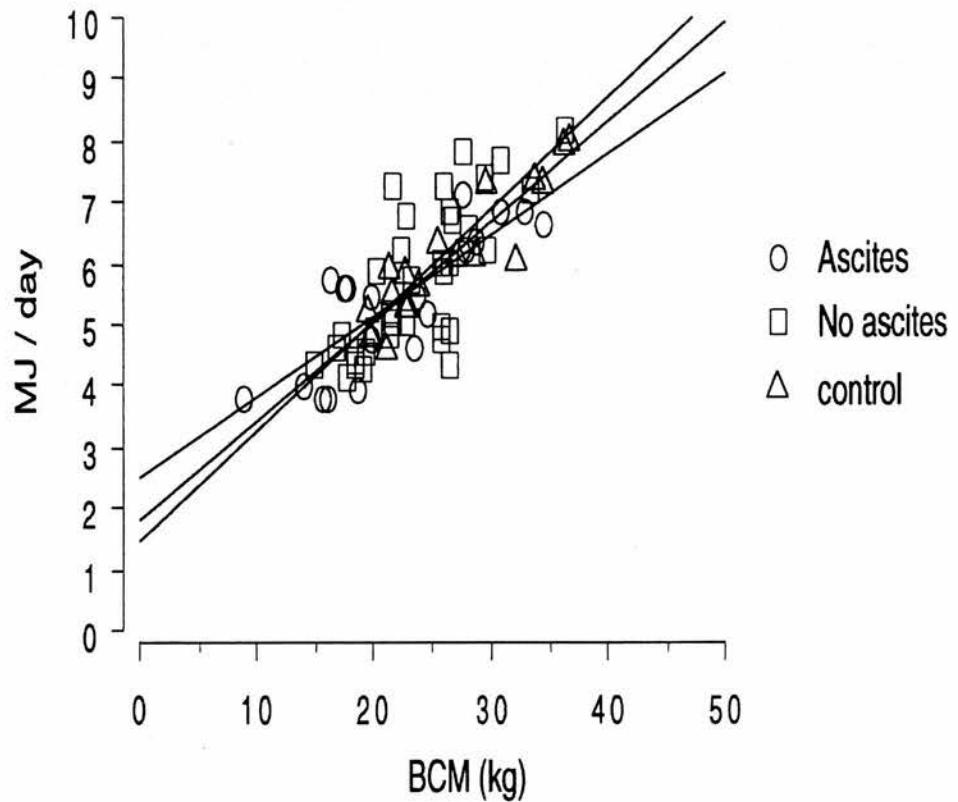
The nutritional, metabolic and clinical status of patients with and without ascites was compared and there were no significant differences between these groups.

### **Cirrhotic Patients Stratified by Disease Severity**

Disease severity may also influence patients nutritional and metabolic status. The nutritional and metabolic status of cirrhotic patients was stratified according to the Child's Pugh score (Table 3.4). Triceps skinfold thickness was significantly lower in patients classified as Child's Pugh C when compared with those patients classified as Child's Pugh A ( $p < 0.05$ ). Serum albumin concentration was significantly lower in patients with Child's Pugh B and Child's Pugh C when compared with the Child's Pugh A group (Child's Pugh

FIGURE 3.2

**The relationship between measured energy expenditure and body cell mass (MFBIA)**



**Control**

$r = 0.957$ ;  $p < 0.0001$      $y = 1.477 + 0.181 * BCM$

**Ascites**

$r = 0.732$ ;  $p < 0.0001$      $y = 2.466 + 0.133 * BCM$

**No Ascites**

$r = 0.809$ ;  $p < 0.0001$      $y = 1.835 + 0.161 * BCM$

**TABLE 3.4 NUTRITIONAL, METABOLIC AND CLINICAL  
STATUS OF CIRRHOTIC PATIENTS CLASSIFIED BY CHILD'S  
PUGH SCORE**

	A (5-6)	B (7-9)	C (10-15)
n	14	26	27
Male : Female	2 : 12	9 : 17	15 : 12
Age (years)	52.9 (2.2)	54.8 (1.7)	55.0 (1.7)
Weight (kg)	63.5 (3.2)	67.4 (2.6)	67.9 (2.9)
Ideal body weight (%)	105 (4.9)	107 (4.8)	109 (4.7)
Recent weight loss (%)	-6 (2.3)	-6 (1.9)	-7 (2.1)
Body mass index	23.7 (1.2)	24.1 (0.9)	24.4 (1.0)
Triceps skinfold thickness (mm)	16.4 (1.7) <sup>a</sup>	12.0 (1.1)	10.7 (1.0)
(%)	102 (10.0) <sup>a</sup>	79 (6.5)	74 (5.8)
Arm muscle circumference (cm)	22.0 (0.6)	22.5 (0.6)	22.6 (0.7)
(%)	94 (2.7)	94 (2.4)	93 (2.9)
Resting energy expenditure (kcal/kg)	20.2 (0.7)	21.1 (0.7)	20.4 (0.7)
Resting energy expend- iture (kcal/kg BCM)	57.2 (7.0)	58.5 (13.2)	54.1 (20.3)
Predicted energy expenditure (%)	91 (2.8)	95 (2.8)	89 (2.6)
Non-protein respiratory quotient	0.82 (0.01)	0.81 (0.01)	0.81 (0.01)
Urinary nitrogen excretion (g/day)	5.7 (0.7)	7.1 (0.9)	5.6 (0.7)
C-Reactive protein (mg/l)	14.2 (6.5)	6.3 (1.3)	12.3 (4.2)

Mean (± SEM)

a = p<0.05 Child's Pugh A versus Child's Pugh C

b = p<0.01 Child's Pugh A versus Child's Pugh C

c = p<0.05 Child's Pugh A versus Child's Pugh B

A versus B  $p<0.05$ ; Child's Pugh A versus C  $p<0.01$ ) but it should be noted that serum albumin concentration is a discriminatory index of Child's Pugh classification. No relationship between disease severity and energy expenditure was observed (Figure 3.1). In this study there were no differences in energy status between cirrhotic patients when classified by the Child's Pugh score.

### **Cirrhotic Patients Stratified by Primary Aetiology**

When patients were sub-divided according to primary aetiology (Table 3.5) patients with biliary cirrhosis (BC) were significantly lighter than either the control or hepatocellular cirrhotic (HC) group (control versus BC  $p<0.001$ ; BC versus HC  $p<0.01$ ). However, when body mass was expressed as ideal body weight or when body mass index (BMI) was calculated no differences were found between patient groups and controls. Triceps skinfold thickness when expressed either as an absolute value or a percentage was significantly lower in the HC patient group when compared with controls ( $p<0.05$ ). In addition, arm muscle circumference was significantly lower in both cirrhotic groups when compared with controls (control versus BC  $p<0.001$ ; control versus HC  $p<0.05$ ) but when expressed as a percentage of standard no differences in AMC between patient groups and control subjects were apparent. There was no relationship between measured energy expenditure and disease severity in either BC or HC patients (Figure 3.1). The NPRQ and urinary nitrogen excretion of patients with HC was significantly lower than control subjects ( $p<0.05$ ).

**TABLE 3.5 NUTRITIONAL, METABOLIC AND CLINICAL  
STATUS OF CIRRHOTIC PATIENTS CLASSIFIED BY PRIMARY  
AETIOLOGY**

	Controls	Biliary Cirrhosis	Hepatocellular
n	18	35	32
Male : Female	9 : 9	3 : 32	23 : 9
Age (years)	50.2 (2.7)	57.6 (1.6)	51.2 (1.7)
Weight (kg)	76.9 <sup>a</sup> (2.9)	62.0 <sup>b</sup> (2.4)	71.9 (2.0)
Ideal body weight (%)	117 (3.5)	106 (4.7)	109 (2.9)
Recent weight loss (%)	0	-7 (1.4)	-6 (2.0)
Body mass index	26 (0.9)	24 (0.9)	25 (0.6)
Triceps skinfold thickness (mm)	15.9 <sup>c</sup> (1.8)	13.5 (1.1)	11.2 (0.9)
(%)	109 <sup>c</sup> (9.2)	83 (6.2)	80 (5.5)
Arm muscle circumference (cm)	25.6 <sup>a,c</sup> (0.7)	21.8 (0.6)	23.1 (0.5)
(%)	102 (3.3)	94 (2.5)	94 (1.8)
Resting energy expenditure (kcal/kg)	19.1 (0.5)	20.6 (1.7)	20.7 (0.5)
Resting energy expend- iture (kcal/kg BCM)	55.8 (1.3)	58.0 (2.5)	61.7 (2.5)
Predicted energy expenditure (%)	95 (2.8)	92 (2.6)	91 (1.9)
Non-protein respiratory quotient	0.84 <sup>c</sup> (0.01)	0.82 (0.01)	0.80 (0.01)
Urinary nitrogen excretion (g/day)	8.6 <sup>c</sup> (0.9)	6.8 (0.7)	5.5 (0.7)
Child's Pugh score	-	8.3 (0.4)	9.0 (0.4)
Serum albumin (g/l)	-	31.6 (0.9)	30 (0.9)
C-Reactive protein (mg/l)	9.4 (5.2)	11.4 (2.7)	8.7 (3.4)

Mean (± SEM) a = p<0.001 controls versus biliary cirrhosis  
b = p<0.05 biliary cirrhosis versus hepatocellular  
c = p<0.05 controls versus hepatocellular

## Orthotopic Liver Transplant Patients

Following OLT, no patient in this cohort ( $n = 23$ ) had clinical evidence of ascites and none continued diuretic therapy after discharge from hospital. The relationship between immunosuppressive therapy and changes in body composition after transplantation will be examined later in this thesis. Characteristics of OLT patients are shown in Table 3.6.

Changes in body weight and arm anthropometry after transplantation are shown in Table 3.7 and MFBIA results are given in Table 3.8. Compared with pre-OLT values, there was a significant increase in body mass (weight, BMI) six months after OLT and this was reflected in increases in arm anthropometry (TSF and AMC), LBM, BCM and fat mass estimated by MFBIA. This increase in body mass continued nine months after OLT and differences were observed between OLT study time points. For example, from three to nine months after OLT (Tables 3.7 and 3.8). These increases in body composition were found in male and female patients where pre OLT weight in females was  $65.4 \pm 3.9$  kg and by 9 months OLT  $71.7 \pm 3.5$  kg ( $p < 0.01$ ). In males average pre-OLT weight was  $76.6 \pm 3.3$  kg and at 9 months OLT this had increased to  $87.7 \pm 3.3$  kg ( $p < 0.05$ ). In the present study, 87% ( $n = 20$ ) of patients nine months after transplantation had a BMI greater than 25, 48% ( $n = 11$ ) and could be classified as being overweight (BMI = 26 - 30) and 39% ( $n = 9$ ) were defined as obese (BMI greater than 30) (Todorovic and Micklewright, 1997).

A significant relationship between measured REE and predicted REE existed at each study time point following OLT (pre-OLT: REE versus predicted,  $r = 0.766$ ,  $p < 0.001$ ; 3 months OLT: REE versus predicted,  $r = 0.775$ ,  $p < 0.001$ ; 6 months OLT: REE versus predicted,



**TABLE 3.6    CHARACTERISTICS OF PATIENTS FOLLOWING  
LIVER TRANSPLANTATION**

Age	53.9 (1.9)
M : F	10 : 13
Primary diagnosis	
biliary cirrhosis	13 1 : 12
hepatocellular cirrhosis	10 9 : 1
Mean ( $\pm$ SEM)	



TABLE 3.8 CHANGES IN BODY COMPOSITION BEFORE AND FOLLOWING LIVER TRANSPLANTATION

	Pre-OLT	3 months	6 months	9 months	Controls
Lean body mass (kg)	48.1 (1.9)	48.8 (2.0)	49.7 (2.0) <sup>a,b</sup>	49.8 (2.0) <sup>c</sup>	49.9 (2.2)
Body cell mass (kg)	24.2 (1.1)	24.5 (1.1)	25.7 (1.1) <sup>d,e</sup>	25.7 (1.2) <sup>a,f</sup>	26.6 (1.4)
Fat mass (kg)	24.5 (1.8)	25.6 (1.3)	28.8 (1.5) <sup>e,g</sup>	30.6 (1.6) <sup>h,i,j</sup>	27.1 (1.5)
mean (± SEM)	a = p<0.05 versus pre-OLT b = p<0.01 versus 3 months OLT c = p<0.05 versus 3 months OLT d = p<0.01 versus pre-OLT e = p<0.0001 versus 3 months OLT		f = p<0.01 versus 3 months OLT g = p<0.0001 versus pre-OLT h = p<0.0001 versus pre-OLT i = p<0.00001 versus 3 months OLT j = p<0.01 versus 6 months OLT		

$r = 0.844$ ,  $p < 0.0001$ ; 9 months OLT: REE versus predicted,  $r = 0.756$ ,  $p < 0.001$ ).

Measured REE was compared with predicted values and significantly lower values in measured energy expenditure was observed at post-OLT time point (pre-OLT  $0.2 \pm 6.1$  MJ/day versus pre-OLT predicted  $6.4 \pm 0.2$  MJ/day,  $p < 0.001$ ; 3 months OLT measured  $6.0 \pm 0.2$  MJ/day versus 3 months OLT predicted  $6.6 \pm 0.2$  MJ/day ( $p < 0.001$ ; 6 months OLT measured  $5.9 \pm 0.2$  MJ/day versus 6 months OLT predicted  $6.7 \pm 0.2$  MJ/day,  $p < 0.0001$ ; 9 months OLT measured  $6.7 \pm 0.2$  MJ/day versus 9 months OLT predicted  $6.7 \pm 0.2$  MJ/day,  $p < 0.001$ ) (Figure 3.3). Compared with pre-OLT values, 43% ( $n = 10$ ) of patients had a greater than 10% decrease in predicted energy expenditure and 22% ( $n = 5$ ) had a greater than 20% decrease in energy expenditure.

## **Discussion**

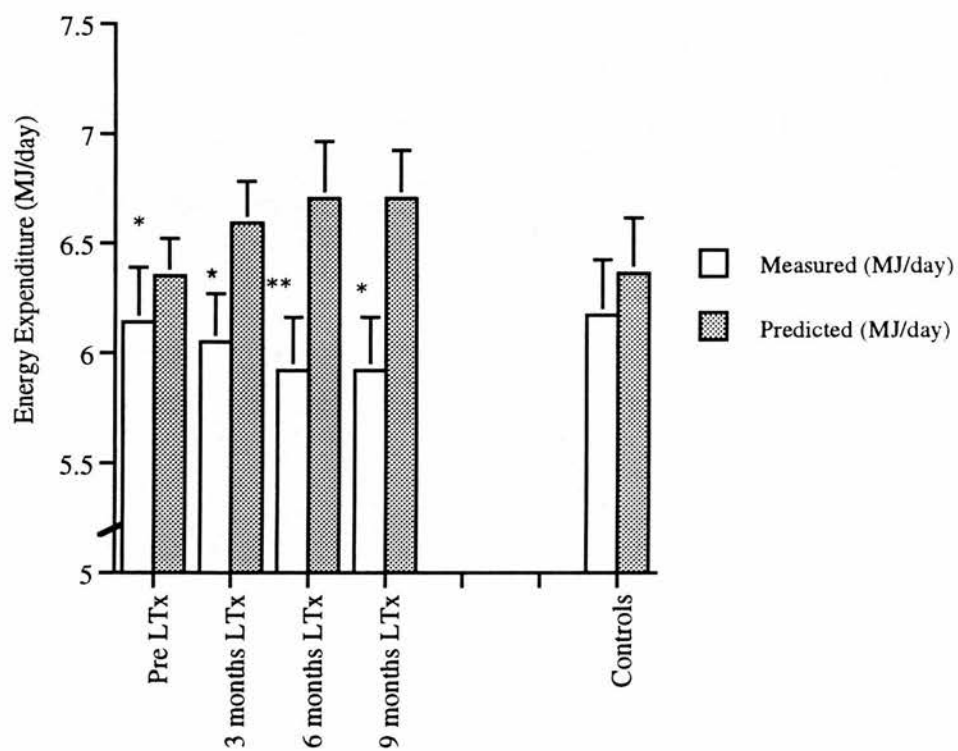
### **Cirrhotic Patients**

In the current study, all patients were elective referrals admitted for consideration of liver transplantation and as such were not septic or had a recent haemorrhage. Indeed, only 40% of this population were stratified as Child's Pugh C whereas 60% presented with Child's Pugh A and B.

### **Cirrhotic Patients Nutritional Status**

Anthropometric measurements are commonly used as practical tools of nutritional assessment. In the present study arm anthropometry was used to estimate fat and muscle reserves as both are considered reliable assessment tools in chronic liver disease (Wicks et al, 1995). Whilst arm muscle area has been considered by some

**FIGURE 3.3 MEASURED ENERGY EXPENDITURE  
VERSUS PREDICTED**



Mean ( $\pm$ SEM)

\* =  $p < 0.001$  versus predicted

\*\* =  $P < 0.0001$  versus predicted

researchers to be a more accurate marker of lean body mass, Thuluvath and Triger (1994) have shown that area measurements are more closely related to radiological assessment of body composition but no difference between area and circumference measurements were apparent when detecting under-nutrition.

In the current investigation, cirrhotic patients had depleted fat and muscle reserves when compared to controls. These observations, combined with lower fasting NPRQ and urinary nitrogen excretion exhibited by this group, suggests a prolonged period of nutrient deprivation. These findings are broadly in line with other studies in patients with cirrhosis where fasting RQ's less than 0.80 have been observed (Owen et al, 1983; Merli et al, 1990; Campillo et al, 1995).

### **Cirrhotic Patients Energy Status**

There were no differences in REE between cirrhotic patients and controls when expressed in relation to body weight, BCM or as a percentage of predictive values. Several research groups have reported hypermetabolism among patients with chronic liver disease (Schneeweiss et al, 1990; John et al, 1990) but considerable debate surrounds the issue of energy status in cirrhosis as other investigators have shown that REE was not significantly different from predictive values (Merli et al, 1990; Owen et al, 1983).

Clearly, it is unreasonable to expect that factors which contribute to energy expenditure will be the same in all cirrhotic patients regardless of the patient stratification employed. In the current investigation predicted energy expenditure was compared

with measured values for cirrhotic patients and 31% (n=21) could be considered as hypometabolic, 63% (n=45) normometabolic and only 6% (n=1) hypermetabolic. This distribution of energy status was almost identical to that found in the control group, where 33% (n=6) were classified as hypometabolic, 61% (n=11) normometabolic and 6% (n=1) hypermetabolic. In addition, the relationship between measured and predicted energy expenditure in patient groups was similar to that found in the control group. It should be noted that the predictive energy equation of Schofield (1985) was derived from a meta-analysis of earlier studies on basal energy expenditure (Harris and Benedict, 1919; Robertson-Reid, 1952; Kleiber, 1932) and the majority of these studies were performed on younger populations. In the present study, 30% of patients and controls were hypometabolic and this may be a result of a reduced body cell mass and increase in fat mass consistent with the natural ageing process. Indeed a study on a large cohort of 123 cirrhotic patients of similar ages, Muller et al (1992) also found that energy expenditure differed from predictive values in half this patient population with 31% classified as hypometabolic and 18% hypermetabolic. Development of a disease specific predictive equation for this population may be of value in estimating energy requirements but given the heterogeneity of this population it may lack sensitivity. This variation in energy expenditure in liver cirrhosis serves to underline the difficulties when making assumptions about energy requirements.

Dolz and colleagues (1991) suggested that the presence of ascites increases whole body energy expenditure in cirrhotic patients. This group performed a study on ten in-patients with alcoholic liver

disease with severe ascites requiring paracentesis. Energy expenditure was measured before and on average 11 days post-paracentesis. Weight loss following drainage averaged 16.6 (range 5.4 - 37.5) kg and was paralleled by a fall in energy expenditure but this decrease could be attributed to an improvement in the patient's general condition. In the present study, no patient's ascites was severe enough to warrant drainage, additionally a strong association between measured energy expenditure and BCM was observed in cirrhotic patients. This finding is supported by a comparative study by Muller et al (1991) which used TBK measurements to determine LBM and compared results with those from single frequency BIA to estimate BCM. They found a highly significant relationship between TBK and LBM and concluded that in mild-moderate ascites (4 litres), BIA remains valid in determining body composition.

The recent introduction of MFBIA offers advantages over their single frequency counterparts where estimates of extra- and intracellular water can be made. Indeed Cha and co-workers (1993) using sodium bromide and tritium isotope dilution techniques and MFBIA demonstrated that in renal patients, MFBIA assisted in the evaluation of water distribution. Whilst further work in this area is required, the use of MFBIA as a bedside tool in the presence of mild/moderate ascites to estimate the metabolically active component of body composition, seems appropriate. Therefore MFBIA determinants of body composition are used in presentation of energy data in the current study.

When patients in the present study were stratified by the presence of ascites, disease severity or primary pathology no



significant differences in energy status between patient groups and controls was observed. It has been suggested that with increasing severity of liver disease, there may be a positive or negative association with oxygen consumption. For example, Schneeweiss et al (1990) in a study of 22 patients with alcoholic cirrhosis, observed an inverse relationship between oxygen consumption and Child's Pugh score. Thus, energy expenditure decreases with increasing disease severity. Conversely, Green et al (1991) in seven PBC patients, observed a positive relationship with oxygen consumption and Child's Pugh score so energy expenditure increased with severity of liver disease. This divergence of results is difficult to reconcile but it is possible that these differences may be related to the presence or lack of an acute phase response in patients admitted for an acute exacerbation of their disease rather than directly to Child's Pugh score. No relationship between energy expenditure and disease severity was observed in the current investigation either in the patient group as a whole or when patients were sub divided by diagnosis.

### **Disease Severity and Nutritional Status**

It is possible that the degree of undernutrition present in a cirrhotic population is associated with disease severity (Mendenhall et al, 1986; Lautz et al, 1992). For example, Muller et al, (1992) showed that Child's Pugh C patients, when compared to Child's Pugh A and B patients were muscle (determined by creatinine excretion) but not fat depleted. However, this finding was not supported by Campillo and colleagues (1997) in a study using anthropometry and creatinine height

index. They showed no differences in nutritional status when patients were stratified using the Child's Pugh score.

It is difficult to understand the differences found in the relationship between disease severity and nutritional status. Although they could be explained by the dynamic nature and weighting of the Child's Pugh scoring system where patients presenting with acute symptoms of their disease will be assigned a high Child's Pugh score. Following resolution of these symptoms, the score could dramatically alter over a period 24 - 48 hours and there would be an apparently dramatic improvement in disease severity. In the present study those patients stratified as Child's Pugh C had reduced fat reserves when compared with Child's Pugh A patients but no other metabolic differences were highlighted. The reason for the significant depletion of fat mass in Child's Pugh C when compared to Child's Pugh A patients remains unclear and did not appear to be related to the aetiology of liver disease (Child's Pugh C: 45% BC, 55% HC). No differences in energy expenditure were highlighted but there was a trend in Child's Pugh C patients toward lower energy expenditure when compared with predictive values or when expressed as a unit of body cell mass. It could be speculated that the significantly depressed fat stores found in the Child's Pugh C group may be a reflection of anorexia and resultant cumulative negative energy balance. As a result, endogenous fat reserves will be drawn upon to meet energy requirements, however, NPRQ in Child's Pugh C patients were not suggestive of an elevated lipid oxidation rate compared with Child's Pugh A and Child's Pugh B. Measurable changes in nutritional indices are only evident after time periods in excess of a week.

The present study showed that Child's Pugh C patients had depressed serum albumin concentrations when compared to patients classified as Child's Pugh B and A. This finding is unsurprising as albumin synthesis rates could be affected by liver dysfunction and depressed serum albumin concentrations in chronic liver disease are not directly related to nutritional state but to disease severity.

### **Primary Pathology and Nutritional Status**

Interestingly, when cirrhotic patients were stratified by primary pathology, patients with HC not only had significantly lower fat stores but their NPRQ and urinary nitrogen excretion was also depressed when compared with controls. The metabolic picture shown by patients with BC suggests these patients have sufficient glycogen reserves available to sustain an overnight fast. Indeed, Di Cecco and colleagues (1989) in a study of 74 liver transplant candidates stratified by disease aetiology, found that patients with BC had improved hepatic synthetic function (biochemical indices) when compared to patients with chronic active and acute hepatitis. This finding supports the hypothesis that metabolic status is related to primary aetiology rather than severity of disease.

### **Cirrhotic Patients - Summary**

This study has shown that cirrhotic patients have depleted energy reserves when compared to a control group of similar age. In the basal state, these patients have a greater reliance on lipid oxidation to meet energy demands which in the absence of an acute phase response serves to conserve muscle mass at the expense of

endogenous fat stores. In cirrhotic patients this is accompanied by a lower urinary nitrogen excretion and it is likely that this is related to preservation of lean body mass. These features of simple starvation (Keys et al, 1950) could be accounted for by the fact that patients in the present study were not recovering from any acute disease related episode. The presence of an acute complication could exert a metabolic effect that would increase gluconeogenesis and in the long term deplete muscle mass. No significant metabolic differences were noted when cirrhotic patients were stratified for the presence of ascites and by Child's Pugh Score.

Irrespective of the limitations of methodologies used to determine nutritional status, undernutrition is prevalent in liver disease (Crawford et al, 1993; Prijatmoko et al, 1993; Greco et al, 1998). Previous research has been driven by investigators who sought to determine the impact of liver disease on nutritional status and the relationship between primary aetiology and disease severity with nutritional and metabolic status (Crawford et al, 1994; Green et al, 1991; Campillo et al, 1997). The importance of studies that examine nutritional depletion in chronic liver disease should not be underestimated as it permits targetting of nutritional support to those most at risk.

An original aspect of the present study is that it has highlighted metabolic differences between cirrhotic patients when stratified by primary aetiology. Only those patients with cirrhosis of hepatocellular origin exhibit a post-absorptive metabolic profile indicative of longterm fasting. This was accompanied by a depletion in the body's energy reserves as reflected in the TSF results of the HC group. When

primary aetiology was considered, it is perhaps not surprising that differences in fasting metabolism and nutritional status have been highlighted. In biliary related cirrhosis, inflammation and liver damage from the biliary canniculi and ducts predominates, whereas the histological profile of alcoholic, cryptogenic and hepatitis C cirrhosis shows intrinsic hepatocellular damage. The hepatocyte is the body's single most important functioning metabolic unit and as a result of extensive hepatic damage, could cause aberrant metabolism that may contribute to the anorexia (Cabre et al, 1990) and consequent loss of body mass found in many cirrhotic patients.

Although it has previously been suggested that the metabolic profile of cirrhotic patients is influenced by primary aetiology (Taylor et al, 1985; Orrega et al, 1987) there are little comparative data on the metabolic and nutritional status of homogeneous, pathology specific groups. Since a focus of this thesis is to examine the role of the liver in ingestive behaviour, the metabolic differences identified between patients with BC or HC are important. The differential effect of pathology may impact on the liver's role as a metabolic sensor and effect changes in ingestive behaviour and body composition.

### **Orthotopic Liver Transplant Recipients**

An increase in body weight following OLT has previously been reported (Palmer et al, 1991; Stegall et al, 1995). In the current study body weight increased by an average of 7.8 kg in the nine months after OLT but may merely reflect weight recovery following a prolonged reduction in energy intake as well as the impact of major surgery. The re-establishment of body composition following a period

of semi-starvation was eloquently demonstrated in The Minnesota Experiment (Keys et al, 1950). Keys work was subsequently revisited by Dulloo et al (1996) who documented that the observed suppression of thermogenesis resulted in accelerated replenishment of body mass and more specifically in fat mass 20 weeks after refeeding (12 weeks on a restricted dietary regimen). Certainly, re-establishing pre-illness or “set-point” body weight could contribute to early post-OLT weight gain. However, given that an individual’s “set-point” for body weight is a tightly regulated system where the average adult’s weight increases by only 10% over two decades (Leibel et al, 1995), the magnitude of weight gain following OLT exceeds pre-illness values.

The present study has shown a significant and rapid increase in adipose tissue rather than lean body mass and it seems unlikely for a number reasons that this is simply a return to “set-point” body weight. Firstly, the duration of weight gain which continues to increase to the nine month study time point rather than four months after refeeding as reported by Keys et al (1950). Secondly, although the nutritional intake of cirrhotic patients may be depleted (Cabre et al, 1998) they are not in an overtly starved state and recent evidence suggests that refeeding after moderate starvation does not disproportionately increase fat mass (Dedrock et al, 1998). Thirdly, in the present study patient’s body weight exceeded reported pre-illness values by  $7.5 \pm 2.1\%$  at nine months. This was significantly higher than the pre-illness values observed six months after OLT and demonstrates that these patients are over-shooting their “set-point” body weight. Whilst criticisms may be levelled at the use of self reported past body weight (pre-illness weight), a recent study by Klipstein-Grobusch et al

(1998) has demonstrated that short and long-term recall of body weight is valid when compared with measured values.

The rapid and dynamic weight gain observed after OLT is an important finding where six patients previously "normally" nourished became overweight and eight patients previously overweight became obese within the study period. Whilst Palmer et al (1991) has shown that pre-OLT overweight patients became more overweight after OLT, this study did not relate post-OLT weight to pre-illness values. Additionally, examination of changes in body composition following OLT have not been considered by previous investigators who have only highlighted increased body mass following liver transplantation. The current longitudinal study permitted OLT subjects to be used as their own controls and, although after OLT lean body mass had stabilised by six months OLT, fat mass continued to increase up to the nine month study time point.

The increase in TSF following OLT suggests an increase in peripheral fat reserves and not a redistribution of fat stores from peripheral to abdominal sites which is characteristic of body composition changes associated with high dose prolonged steroid therapy (Horber et al, 1986). No other comparable body composition studies on OLT patients are available. However, Steiger et al (1995) studied kidney transplanted patients and found that fat mass increased by 4.9 kg in males and in females 0.1 kg over a 16 month period and is considerably less than that found in the current study. Furthermore, the level of immunosuppressive therapy following OLT is normally substantially lower than that prescribed to renal recipients. However, comparisons between the nutritional status of renal transplant recipients and OLT patients have not been performed but does suggest



that absolute amounts of immunosuppressive drugs may not be predictive of weight change following OLT.

### **Summary OLT Patients**

Results of the current study suggest there is a failure of regulatory systems that control body weight and this is particularly worrisome given the susceptibility of the patient undergoing liver transplantation to develop metabolic complications which may be further exacerbated in the presence of excessive weight gain. Clearly elucidation of the mechanisms involved in post-OLT weight gain has important implications for the longterm management of these “at risk” patients. Long term energy balance is tightly controlled (Leibel et al, 1995) and small deviations from “normal” in any single component of energy metabolism (dietary intake, exercise, thermogenesis, basal energy expenditure) will in the long term result in changes in body mass. In the current study there was a significant decrease in energy expenditure at each post-OLT study time point when compared with predictive values. However, this finding may merely reflect a disproportionate rise in fat compared to lean body mass following OLT. This decrease in REE will be explored more fully in the next chapter (Chapter 4).

An uninvestigated dimension of OLT weight gain is the influence of denervation and loss of hepatic neural signalling to the brain stem. It could be postulated that central effector pathways may induce suppression of energy expenditure. A decrease in basal energy expenditure is a phenomenon associated with starvation and if latent signalling through hepatic afferents is absent it may be interpreted by central controllers of ingestive behaviour as nutrient deprivation.



## CHAPTER 4

### DIET INDUCED THERMOGENESIS IN LIVER CIRRHOSIS AND IN LIVER TRANSPLANT RECIPIENTS

Loss of body mass in liver cirrhosis has been widely reported (Merli et al, 1990; Prijatmoko et al, 1993; Greco et al, 1998). In order for changes in body composition to occur there may be alterations in, energy intake, resting energy metabolism, physical activity and/or diet induced thermogenesis. The resting energy profile of cirrhotic patients and liver transplant recipients was described in Chapter 3. Generally, patients with liver cirrhosis lead a sedentary lifestyle, therefore it seems unlikely that increased activity levels would contribute to loss of body mass. It is not unreasonable to suggest that there may be changes in dietary thermogenesis in liver disease where loss of body mass, impaired liver function and altered humoral responses could affect the metabolic response to feeding.

Only a handful of metabolic studies have considered the effect of feeding in liver cirrhosis. These investigations have predominantly been performed in small groups of patients presenting with alcohol related disease and have produced conflicting results suggesting no change (Eriksson et al, 1989) or a blunting in the thermic response to feeding (Campillo et al, 1992). The magnitude of any change in thermogenesis in cirrhotic patients is of quantitative importance in energy balance and the longterm regulation of body mass.

In contrast with the pre-operative situation where weight loss predominates, patients who undergo liver transplantation have been shown to gain weight progressively for at least two years after surgery (Davidson et al, 1998). It is possible that a reduction in dietary induced thermogenesis might contribute to such weight gain.

This study considered the effect of a mixed meal on energy expenditure in both stable cirrhotic patients and in patients submitted to OLT.

## **Patient and Methods**

### **Patients**

The sex, age and BMI of the 36 cirrhotic patients were randomly selected to enter the test meal limb of the study (Table 4.1). Twenty-three of the 36 patients underwent OLT. Following OLT, five patients sustained complications (1 deep vein thrombosis; 1 hepatic duct stenosis; 1 biliary stricture; 2 long-term sepsis) which did not permit them being studied further. Therefore data from the remaining 18 OLT patients was analysed in the pre-operative test meal limb of the study and compared with data obtained at 3, 6 and 9 months postoperatively. Eighteen healthy volunteers were also recruited and studied on a single occasion.

### **Methods**

To determine the thermogenic response to feeding a test meal (10% protein, 31% fat and 59% carbohydrate) was prescribed at 15 kJ/kg body weight and consumed within five minutes. Indirect calorimetry was used

to measure energy expenditure at study time points shown in Figure 2.1. The thermogenic effect of the meal was calculated and results expressed as a percentage above baseline fasting values. The integrated thermic response to feeding was determined from the area under the curve (AUC) which was calculated using the trapezoid method and expressed as arbitrary units. The methods used are described in detail in Chapter 2.

## Results

### Cirrhotic Patients

Characteristics of the cirrhotic patient population and control group are shown in Table 4.1. As might be expected, there was no significant difference in the energy value of the test meal in relation to measured resting energy expenditure between cirrhotic patients and control subjects (cirrhotics:  $18.3 \pm 0.4\%$ ; controls:  $18.9 \pm 0.4\%$ ) or when patients were stratified for disease severity (Child's Pugh A:  $18.6 \pm 0.6\%$ ; Child's Pugh B:  $17.9 \pm 0.5\%$ ; Child's Pugh C:  $18.8 \pm 1.2\%$ ) or primary aetiology (BC:  $18.9 \pm 0.8\%$ ; HC:  $18.0 \pm 0.5\%$ ).

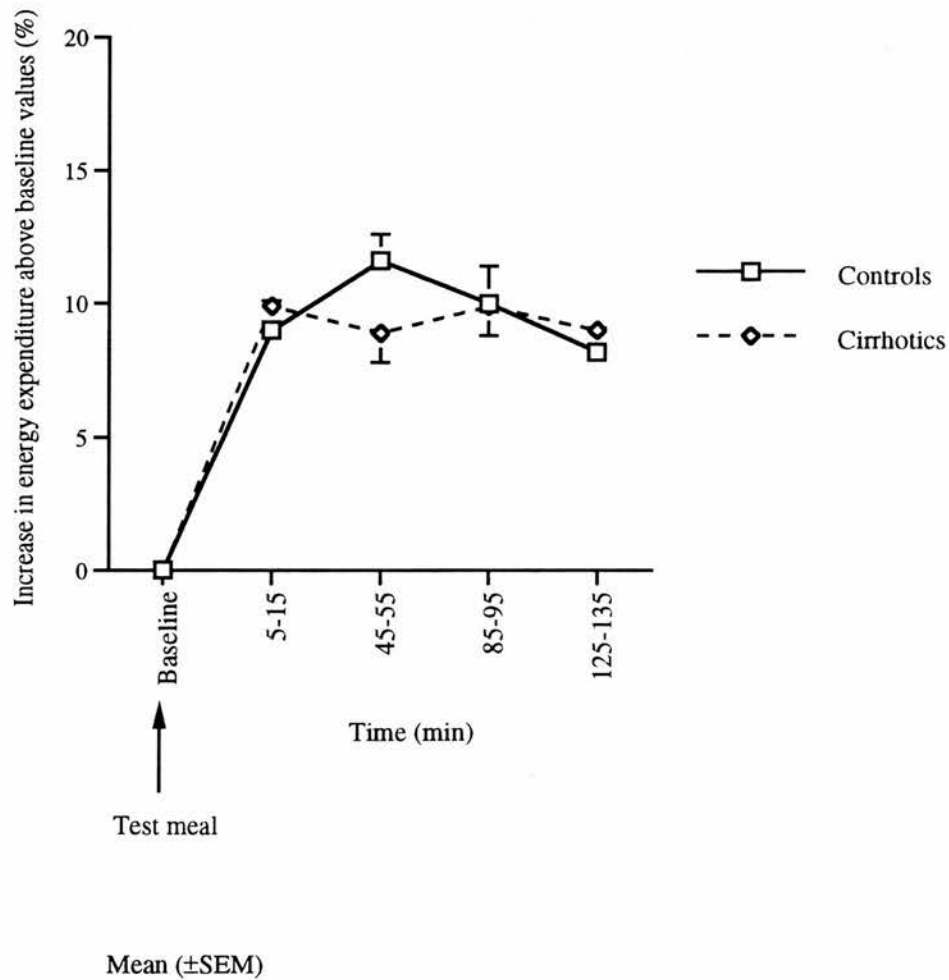
No overall differences in the thermic effect of feeding was observed when all 36 cirrhotic patients were compared with the healthy controls (Figure 4.1). However, when cirrhotic patients were stratified according to the modified Child's Pugh score, the change in energy expenditure from baseline values was significantly higher at the first post prandial time point (5-15 min) in Child's Pugh A patients when compared with Child's Pugh C patients (Child's Pugh A:  $14.1 \pm 2.4\%$  versus Child's Pugh C:  $6.1 \pm 1.3\%$ ,  $p < 0.05$ ). There was a trend for diet induced

**TABLE 4.1 CHARACTERISTICS OF CIRRHOTIC PATIENTS  
AND CONTROL SUBJECTS**

	n	Sex (M : F)	Age (years)	BMI	Resting Energy Expenditure (kcal/kg BCM)
Controls	18	9 : 9	50.2 (2.7)	26.4 (0.9)	56.2 (1.2)
Cirrhotic patients	36	15 : 21	53.4 (1.6)	24.6 (0.8)	59.2 (1.6)
Child's Pugh A	10	2 : 8	54.0 (2.9)	24.1 (1.4)	58.9 (2.1)
Child's Pugh B	16	7 : 9	53.6 (2.4)	23.8 (0.9)	58.0 (1.7)
Child's Pugh C	10	6 : 4	52.2 (3.3)	21.6 (2.1)	55.3 (1.8)
Biliary cirrhosis	18	0 : 18	55.9 (1.7)	23.8 (1.3)	60.1 (2.0)
Hepatocellular cirrhosis	18	15 : 3	50.9 (2.5)	25.5 (0.8)	58.1 (2.4)

Mean ( $\pm$  SEM)

**FIGURE 4.1    THERMOGENIC RESPONSE TO FEEDING  
IN CONTROL SUBJECTS AND CIRRHOTIC  
PATIENTS**

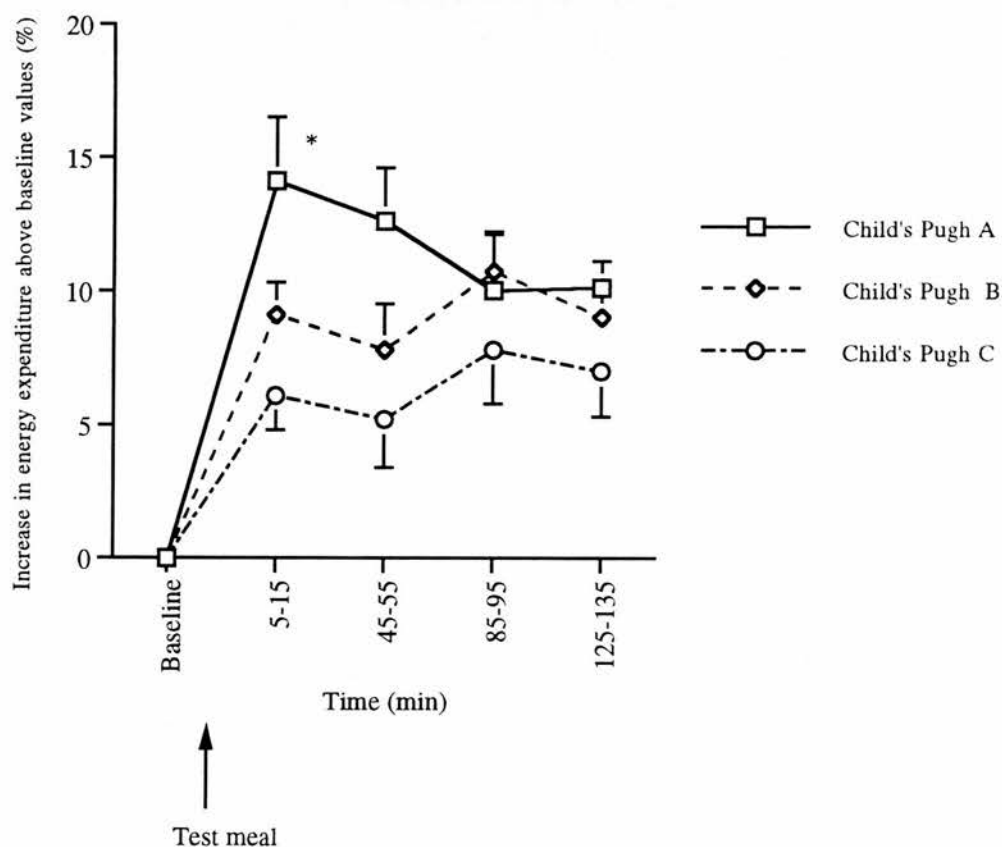


thermogenesis in Child's Pugh A patients to be higher than Child's Pugh B and C patients up to 85 minutes following meal ingestion. In Child's Pugh B and C patients the diet induced thermogenic response curves were similar. The integrated thermic response (AUC) was greater in the Child's Pugh A group compared with Child's Pugh C patients (Child's Pugh A:  $1543 \pm 140.6$  AUC versus Child's Pugh C:  $835 \pm 204.8$  AUC,  $p < 0.05$ ) (Figure 4.2).

When patients were stratified by primary aetiology, the thermic response was significantly higher in cholestatic (BC) patients when compared with HC patients (BC:  $12.7 \pm 1.5\%$  versus HC:  $7.0 \pm 1.0\%$ ,  $p < 0.05$ ) at the first time point. In addition, the integrated meal response was also greater in the BC patients when compared with the HC group (BC:  $1472 \pm 123.6$  AUC versus HC:  $984 \pm 137.1$  AUC,  $p < 0.05$ ) (Figure 4.3).

When patients were stratified by Child's Pugh score, seven out of the ten Child's Pugh A patients had cholestatic disease. Given this potential source of bias primary aetiology was used when examining oxygen consumption in response to feeding. No differences in the integrated response between BC, HC and control subjects was observed (Figure 4.4). There was an early increase in oxygen consumption in BC patients which was marginally significant ( $p = 0.06$ ) when compared with the HC group. This increase in oxygen consumption was not indicative of substrate utilisation as shown by NPRQ (Figure 4.5).

**FIGURE 4.2 THERMOGENIC RESPONSE TO FEEDING  
IN CIRRHOTIC PATIENTS STRATIFIED  
BY DISEASE SEVERITY**

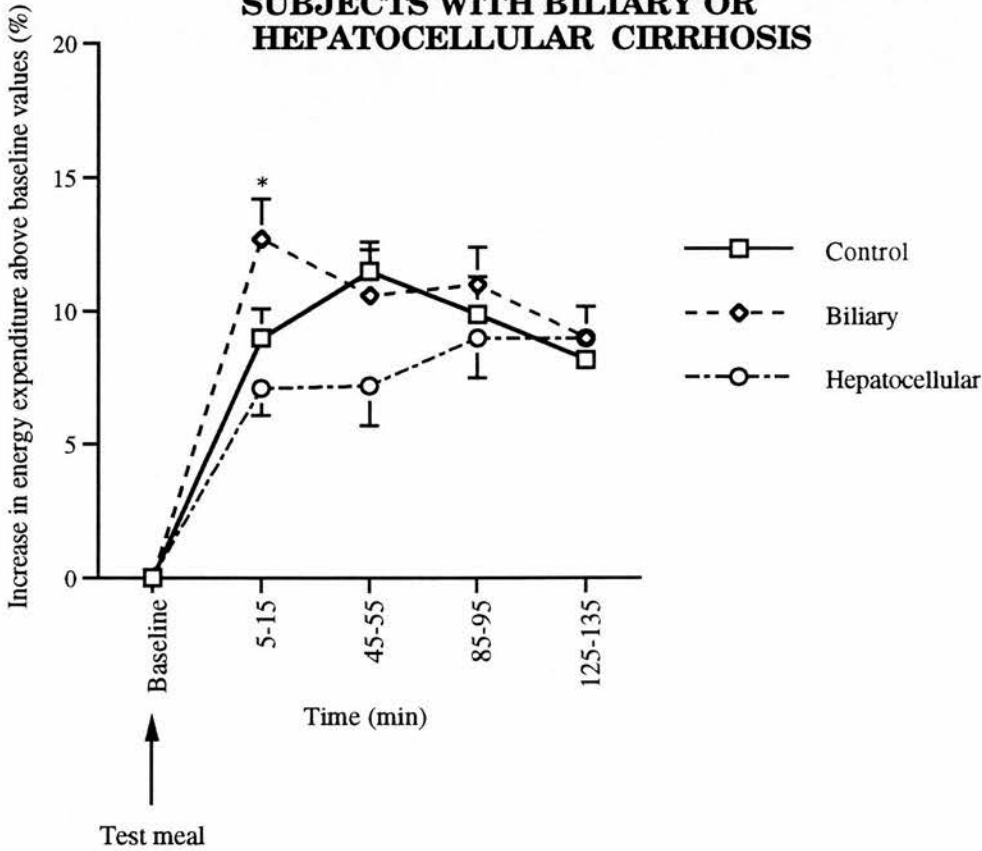


Mean ( $\pm$ SEM)

\* =  $p < 0.05$  Child's Pugh A versus Child's Pugh C

AUC =  $p < 0.05$  Child's Pugh A versus Child's Pugh C

**FIGURE 4.3 THERMOGENIC RESPONSE TO FEEDING  
IN CONTROL SUBJECTS AND CIRRHOTIC  
SUBJECTS WITH BILIARY OR  
HEPATOCELLULAR CIRRHOSIS**



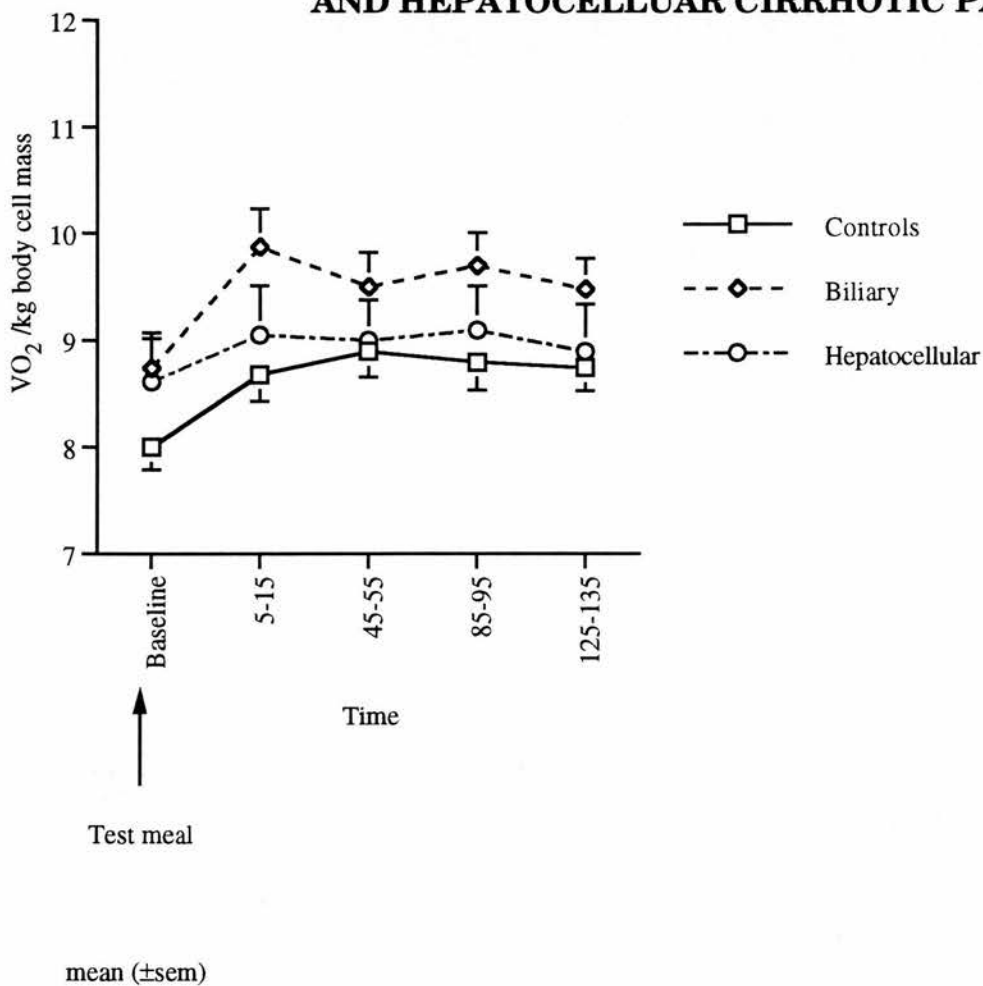
Mean ( $\pm$ SEM)

\* =  $p < 0.05$  biliary versus hepatocellular

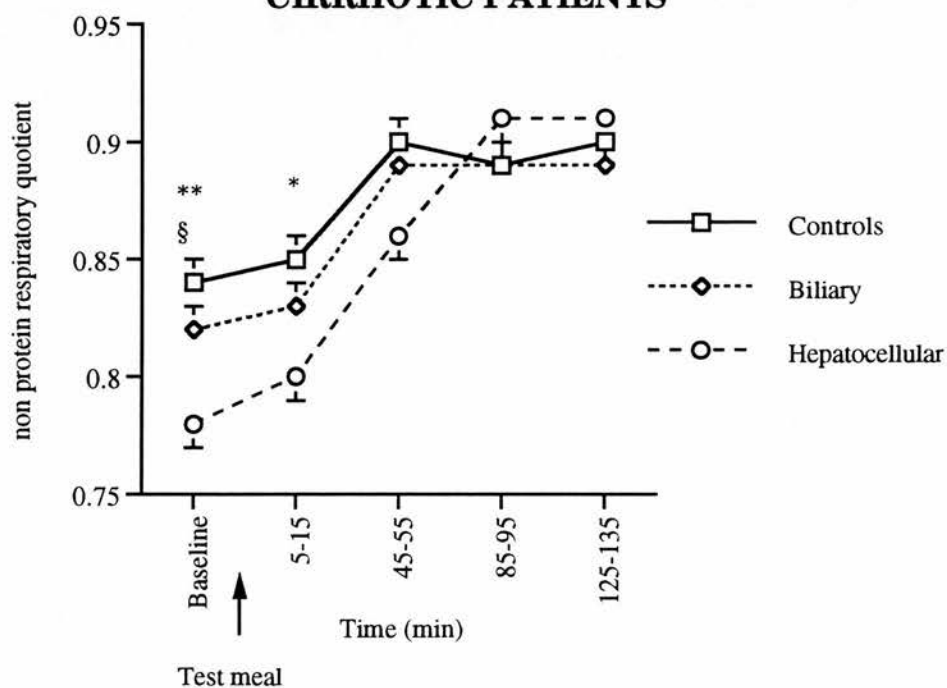
AUC =  $p < 0.05$  biliary versus hepatocellular



**FIGURE 4.4 OXYGEN CONSUMPTION IN RESPONSE TO FEEDING IN CONTROL SUBJECTS, BILIARY AND HEPATOCELLULAR CIRRHOTIC PATIENTS**



**FIGURE 4.5 RESPIRATORY QUOTIENT IN RESPONSE TO FEEDING IN CONTROL SUBJECTS, BILIARY AND HEPATOCELLULAR CIRRHOTIC PATIENTS**



mean (sem)

\*\* =  $p < 0.0001$  controls versus hepatocellular cirrhosis

§ =  $p < 0.001$  biliary versus hepatocellular cirrhosis

\* =  $p < 0.05$  controls versus hepatocellular

## OLT Patients

The response to feeding was studied in seven male and 11 female patients (mean age  $52.8 \pm 2.4$  years) following OLT. All ten BC patients were female and seven of the eight HC patients were male. Following OLT, no significant changes in the thermogenic response (% energy above baseline values) to feeding were observed (Figure 4.6). Furthermore, after transplantation pre-OLT pathology had no differential effect on thermogenesis.

At each study time point OLT resting energy expenditure (per unit body cell mass) and the response to feeding was also examined. A progressive decrease in REE occurred after OLT which reached significance nine months after liver transplantation. In addition, the calculated area under the curve when compared with pre-OLT values were significantly lower at 9 months OLT (Table 4.2).

A comparison of the energy response to feeding between control and OLT patients suggested that the postprandial energy response to feeding was lower 9 months after OLT when compared with controls at 5-15, 85-95 and 125-135 minutes after meal ingestion. Baseline values 9 months after OLT tended to be lower than those observed in controls ( $p=0.06$ ). No significant differences in the AUC between control subjects and post-OLT patient groups were observed.

**FIGURE 4.6 THERMOGENIC RESPONSE TO FEEDING IN PATIENTS BEFORE AND FOLLOWING TRANSPLANTATION**

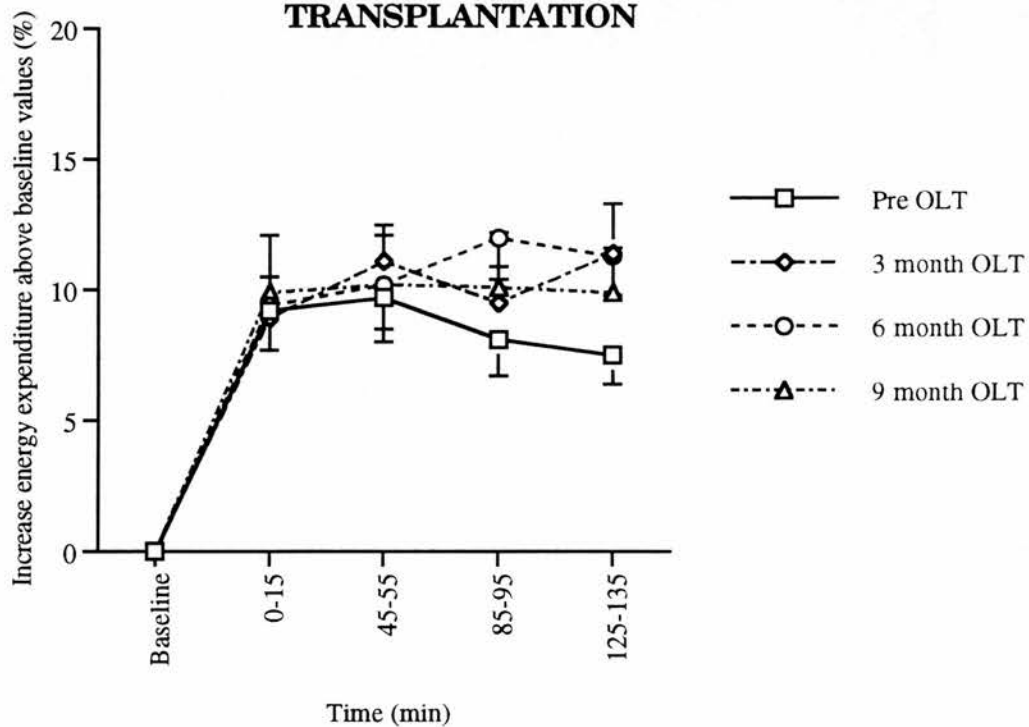


TABLE 4.2 ENERGY EXPENDITURE IN RESPONSE TO FEEDING PRE- AND POST-LIVER TRANSPLANTATION  
(kcal/kg BCM)

	Baseline	5-15 mins	45-55 mins	85-95 mins	125-135 mins	AUC
Pre-OLT	60.3 (1.6) <sup>a</sup>	65.9 (1.9) <sup>b</sup>	65.7 (1.7) <sup>a,b</sup>	65.0 (1.7)	64.9 (1.8)	8750 (219) <sup>a</sup>
3 months OLT	58.2 (1.8)	64.1 (2.2) <sup>c</sup>	64.8 (2.2) <sup>c,d</sup>	64.5 (2.4)	65.7 (2.5) <sup>c,d</sup>	8683 (408)
6 months OLT	56.2 (1.3)	61.4 (1.8)	61.3 (1.7)	62.3 (1.8) <sup>e</sup>	62.5 (1.7)	8129 (264)
9 months OLT	53.7 (2.2)	59.4 (2.1)	59.3 (2.1)	58.0 (2.1)	59.0 (2.0)	7798 (308)
Control	59.2 (1.9)	67.2 (2.2) <sup>c,e</sup>	65.3 (2.2)	66.7 (2.1) <sup>e</sup>	65.2 (1.9) <sup>e</sup>	8417 (238)
Mean (± SEM)	a = p<0.05 versus 9 months OLT b = p<0.05 versus 6 months OLT c = p<0.05 versus 6 months OLT		d = p<0.01 versus 9 months OLT e = p<0.05 versus 9 months OLT			AUC = area under curve

## Discussion

### Cirrhotic Patients

In liver cirrhosis, the influence of the thermic effect of food and its impact on energy balance remains ambiguous (Eriksson et al, 1991; Green et al, 1991; Campillo et al, 1992). The current study examined the thermic effect of a mixed meal (15 kJ/kg body weight; 59% carbohydrate, 31% fat, 10% protein) in 36 cirrhotic patients and is to date the largest cohort studied.

It has been suggested that antecedent diet may influence measurements of metabolic rate (Dauncey; 1980) but this remains an area of contention. Whilst Dauncey (1980) suggests that the previous day's dietary intake influences metabolic rate for at least 14 hours after the last meal, this finding has not been consistently replicated by other investigators (Stock, 1980; Lammert et al, 1987). In the current study, due to the clinical and practical constraints imposed it was not possible to control diet prior to the study and therefore the influence of this factor cannot be resolved.

The liquid test meal in the present study was used to facilitate gastric emptying and permit the peak meal response to be observed within a two hour post-prandial period. All subjects were investigated over a four hour period and when questioned, patients felt that any time extension to this study would prove too onerous. This caveat was particularly important for patients entered into the longitudinal limb of the study.

In the current study the energy content of the test meal was related to body weight. It has previously been suggested that test meals should be related either to body weight (Sharief & Macdonald, 1982) or to resting energy expenditure (Segal et al, 1990). Previous investigators have used either a standard meal (unrelated to body weight) (Eriksson et al, 1989) or related meal size to body weight (Green et al, 1991; Campillo et al, 1992). In the present study no significant difference in the energy value of the test meal when prescribed in relation to resting energy expenditure was found between cirrhotic patients and control subjects.

There was no difference in the thermic response to feeding when cirrhotic patients were compared with control subjects. When patients were stratified by disease severity an early and significant increase in energy expenditure in Child's Pugh A patients ( $p < 0.05$ ) was observed although this was also reflected in the response elicited by BC patients. Seven of the ten patients presenting with Child's Pugh A cirrhosis had cholestatic disease and it seems reasonable to suggest that the early increase in thermogenesis is related to aetiology rather than severity of liver disease.

The majority of patients with Child's Pugh C cirrhosis presented with HC disease (seven out of nine, 78%). In comparison to BC patients, the HC group had a blunted thermogenic response. Alternatively it could be said that BC patients had a significantly higher thermogenic response than their HC counterparts. Despite these differences neither patient group's thermogenic response was significantly different from control subjects. Green et al (1991) in a study of seven BC patients

observed a significant increase in the thermogenic response to a test meal in BC patients when compared to controls. These patients were studied over a four hour post-prandial period and the test meal was three times larger than that administered in the present study. In addition, the time allowed for consumption of the test meal by Green and colleagues (1991) was 45 minutes compared with 5 minutes in the present study. Furthermore, calorimetry was first performed 30 minutes after meal consumption and as a result the early and significant post-prandial increase in thermogenesis (5 - 15 minutes) seen in the current study would be missed. Given the significant differences in study design, direct comparisons between these two studies are difficult.

This early increase in oxygen consumption seen in response to feeding in BC patients is not seen in the HC patient group and it is unlikely that utilisation of the meal would be evident five minutes after ingestion. This is confirmed by the NPRQ results where no early increase in respiratory quotient was observed in BC patients and does not infer meal utilisation at this time point. The reason for this early increase in thermogenesis seen in BC patients cannot be fully explained from present results and would require determination of humoral events, sympathetic nervous system activity as well as the rate of gastrointestinal nutrient uptake. Though speculative it could be that the early thermogenic response observed in BC patients may be an adaptive response acting to offset the effects of insidious fat malabsorption through maximising nutrient absorption by active transport mechanisms. In other words, when a meal enters the gastrointestinal tract there may be an increase in



both obligatory and facultative thermogenesis, the former reflecting enhanced transport mechanisms and the latter up-regulation of the sympathetic nervous system. This early increase in thermogenesis is transient and at the 45 minute post-prandial time point, the thermic response to the meal in BC patients is similar to control subjects. To date no studies have examined facultative thermogenesis in BC patients but given that diet induced thermogenesis in BC patients was not significantly different from control subjects it is unlikely to contribute to long term changes in energy balance. Indeed, nutritional assessment data in BC patients has shown that nutritional status is well preserved in this group (see Chapter 3).

The thermogenic response of HC patients tended to be lower than controls but did not reach statistical significance, either in terms of time point comparisons or the integrated meal response. This finding is supported by the work of Campillo et al (1992) who also observed a depression of dietary induced thermogenesis in HC patients but the effect was not different to that seen in control subjects. In addition, Campillo et al (1992) examined the humoral response to feeding including catecholamine measurements in HC patients. Paradoxically, although this group observed an increase in sympathetic tone during feeding (increased noradrenaline), there was a blunting of thermogenesis in HC patients. This group concluded that insulin resistance could be implicated in the blunting of the thermogenesis. However, this is clearly an oversimplification of a complex phenomenon where, for example, counter regulatory hormones such as glucagon play an important role in

metabolic regulation. Moreover, Campillo et al (1992) observed no correlation between noradrenaline levels and metabolic rate, suggesting that the apparent thermogenic blunting found in HC cirrhosis cannot be attributed to any single factor.

In the present study, the blunting of the thermogenic response seen in HC patients when compared with BC patients may, in the long term, exert an energy sparing effect in a population who have depleted endogenous energy reserves (see Chapter 3). To fully understand the importance of this differential effect related to thermogenic response and aetiology, further studies are required that examine concurrent humoral events as well as the thermogenic response to feeding.

In summary, the current study has generated important and original findings. In particular the early thermogenic peak in patients with BC disease has not previously been reported and warrants further investigation. Additionally, the difference in thermogenesis between patients with BC and HC cirrhosis suggests their metabolic response to feeding is different. Neither patient group's thermogenic response was different to controls but if the blunting of the thermogenic response in the HC patients is considered cumulatively, this may exert an energy sparing effect. It is unlikely that the early and transient increase in thermogenesis would have a cumulative effect on energy balance in BC patients given their preserved nutritional status (see Chapter 3).

## OLT Patients

Few studies have considered the metabolic effects of feeding in OLT patients (Green et al, 1991). None have considered that the loss of hepatic afferent innervation could have an effect on energy metabolism which in the longterm may impact on nutritional status.

In the current study, no significant differences in the thermogenic response to feeding was seen after transplantation. In patients followed up to nine months following transplant, the thermogenic response to feeding mirrored that found in control subjects. An important finding in OLT patients was the decrease in REE initially highlighted in Chapter 3. There was a sequential decrease in REE at each three monthly OLT time point, reaching significance six months after transplantation. This reduction in REE also resulted in a significantly lower integrative energy response (AUC) at nine months after OLT when compared to pre-transplant values. The post prandial response to feeding nine months after OLT was lower than controls at the 5-15, 85-95 and 125-135 minutes study time points (Table 4.2).

Nine months after transplantation, there was an 11% decrease in REE compared to pre-OLT values. This extrapolates to an energy economy of approximately 20 MJ in three months (approximately 0.75 kg fat plus associated lean tissue; Mela and Rogers, 1998). Clearly this is an over-simplification of energy dynamics and its subsequent translation to the impact on body energy reserves but it does contextualise the effect of down regulation of energy metabolism. Obviously other factors such as dietary intake and activity levels, which normally account for some 30% of

total energy expenditure (Ravussin and Bogardus, 1989) will also contribute to the energy balance equation.

In the current study activity levels were not determined but it should be highlighted that lethargy is one of a number of established indications for OLT. However, 83% of transplanted patients had returned to employment implying that following OLT patients activity levels increase and would consequently result in a rise in REE. Given that OLT activity levels have increased, then the energy economy elicited in these patients following OLT is in fact more pronounced than observed.

Interestingly, in the only other metabolic study on pre- and post OLT patients, Green et al (1991) examined the energy response to feeding. They noted that REE in patients was significantly lower in BC patients seven months following transplantation when compared with pre-OLT BC patients. The discussion in Green and colleagues paper (1991) focused on the hypermetabolism of the BC patients rather than what may have been the hypometabolism of the post-OLT patients. Nevertheless the fall in energy expenditure seen in these OLT patients does support the reduction in REE evident in the present longitudinal study.

If the mechanisms involved in post-OLT weight gain are to be elucidated, the reason for the down-regulation of energy metabolism following transplantation requires further investigation. Resting energy expenditure includes that required to drive various metabolic processes such as protein turnover, substrate transport recycling, ion gradient maintenance, heart and diaphragm contraction. All of these processes may be involved in changes in resting metabolic rate but to date the exact

mechanisms involved in down-regulation of energy metabolism are not fully understood.

In OLT patients appropriate integration of metabolic information in the hypothalamus will be lost. It could be hypothesised that the lack of information relating to fuel oxidation and satiety signalling may mean that the brain stem assumes that the body is undergoing a period of energy restriction and as a consequence reduces REE in an attempt to conserve body mass. Rather than weight loss, these patients are undergoing a period of rapid weight gain reflected in an increase in fat mass rather than lean tissue (Chapter 3). Normally, a rise in fat mass should result in an increase in circulating levels of the hormone leptin produced in the adipocyte which acts to increase energy expenditure and reduce energy intake (Zhang et al, 1994). Leptin has been shown to be pivotal in energy balance (Campfield et al, 1996) although circulating leptin levels have not been examined in OLT patients but their determination may add to the understanding of post OLT energy economy.

In summary, this is the first study in OLT patients that has examined the energy response to feeding in the fasted and fed state. The observed energy conservation in the presence of increasing body mass may be a result of metabolic and splanchnic bed denervation resulting in absence of the integrative control of energy balance.

## CHAPTER 5

### FASTING SUBSTRATE UTILISATION IN LIVER CIRRHOSIS AND LIVER TRANSPLANT RECIPIENTS

In chronic liver disease, disturbances in fasting nutrient metabolism were first documented some 15 years ago (Owen et al, 1983). The increase in fat oxidation observed in cirrhotic patients following an overnight fast was comparable to that found in normal subjects after a 72 hour fast (Romijn et al, 1991). However, most studies that have examined fasting metabolism in cirrhotic patients have been performed in HC patients recovering from an acute complication of their liver disease. This study examined fasting substrate oxidation rates in stable cirrhotic patients and in a sub-group of patients following OLT.

#### Patients and Methods

##### Patients

Patients were studied in the fasting state and are fully described in Chapter 3. Sixty-seven cirrhotic patients with histologically proven cirrhosis were recruited (Table 3.1). Twenty three of these patients underwent liver transplantation and were studied at three, six and nine months after discharge from hospital. A group of 18 healthy controls were also recruited (see Chapter 3).

## Methods

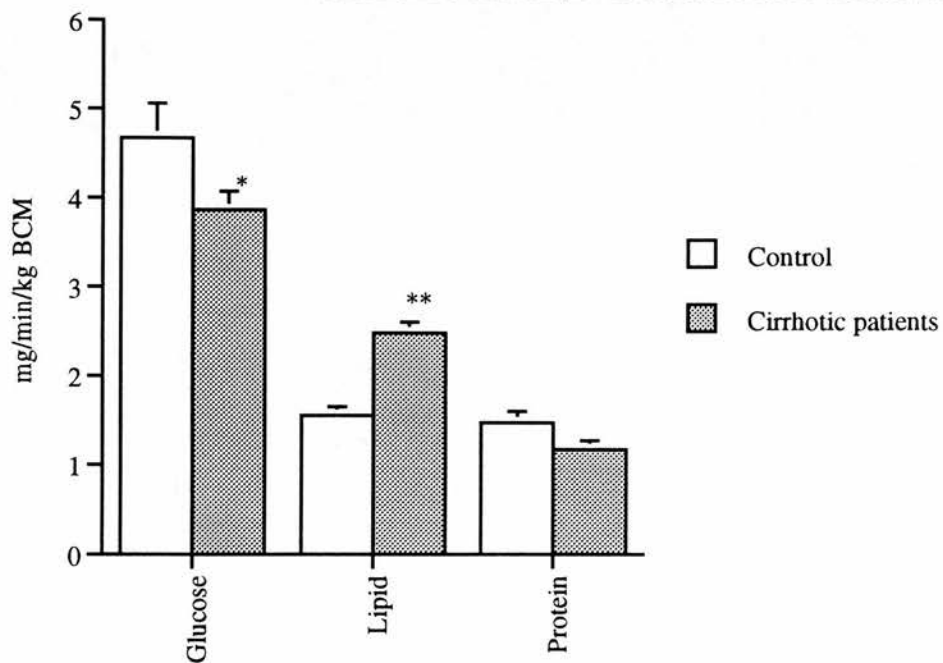
Following an overnight fast indirect calorimetry was used to measure subjects oxygen consumption and carbon dioxide production. In addition urinary nitrogen excretion was measured from subjects 24 hour urine collection. These physiologic measurements allowed calculation of fasting substrate oxidation rates. Methods and calculations used to determine NPRQ and substrate oxidation rates are shown in Chapter 2.

## Results

### Cirrhotic Patients

Demographic and clinical details of cirrhotic patients are shown in Table 3.1. Fasting NPRQ was significantly lower in cirrhotic patients when compared with control subjects (controls:  $0.85 \pm 0.01$  versus cirrhotics:  $0.81 \pm 0.01$ ,  $p < 0.001$ ). The lower NPRQ exhibited by cirrhotic patients when compared with controls was reflected in an increased rate of lipid oxidation and decreased rate of glucose oxidation (Lipid: controls:  $40.9 \pm 3.6$  mg/min versus cirrhotics:  $54.9 \pm 2.2$  mg/min,  $p < 0.01$ ; Glucose: controls:  $120.8 \pm 10.7$  mg/min versus cirrhotics:  $86.7 \pm 3.8$  mg/min,  $p < 0.001$ ). Given the gender bias in this patient population post-absorptive substrate oxidation rates in control subjects and cirrhotic patients are presented per unit of BCM to normalise data (Figure 5.1). Rates of glucose oxidation were significantly lower and fat oxidation significantly higher in cirrhotic patients compared to control subjects (Glucose: control:  $4.67 \pm 1.6$  mg/kg BCM versus cirrhotics:  $3.86 \pm 1.50$ ,  $p < 0.05$ ;

**FIGURE 5.1 SUBSTRATE OXIDATION IN CONTROL SUBJECTS AND CIRRHOTIC PATIENTS**



Mean ( $\pm$  SEM)

\* =  $p < 0.05$  versus controls

\*\* =  $p < 0.001$  versus controls



Lipid: control:  $1.55 \pm 0.4$  mg/kg BCM versus cirrhotics:  $2.48 \pm 0.9$  mg/kg BCM,  $p < 0.001$ ).

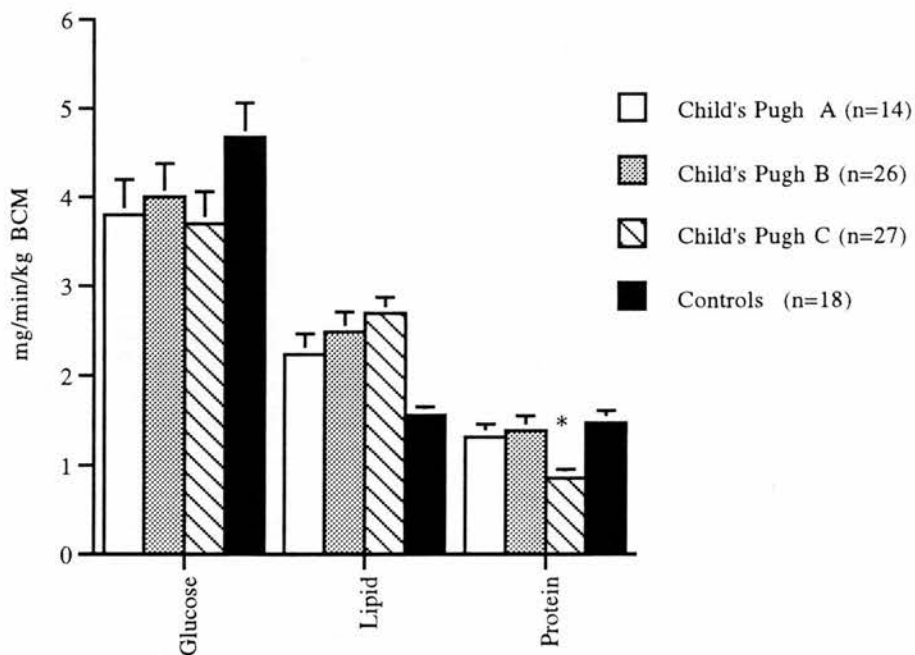
### **Cirrhotic Patients Stratified by Disease Severity**

Demographic and clinical details of patients stratified by disease severity are shown in Table 3.4. When patients were classified using modified Child's Pugh score no difference in NPRQ or in fasting oxidation rates of glucose or lipid were observed (Figure 5.2). Protein oxidation rates were significantly lower when Child's Pugh C cirrhotic patients were compared with the Child's B Pugh group (Child's Pugh B:  $1.39 \pm 0.23$  mg/kg BCM versus Child's Pugh C:  $0.80 \pm 0.1$  mg/kg BCM,  $p < 0.05$ ).

### **Cirrhotic Patients Stratified by Primary Aetiology**

Demographic and clinical details of patients stratified by primary aetiology are shown in Table 3.5. Non protein respiratory quotient was significantly lower in HC patients when compared with control subjects (controls:  $0.85 \pm 0.01$  versus HC:  $0.79 \pm 0.01$ ,  $p < 0.0001$ ). Additionally, NPRQ was significantly lower in HC patients than in the BC patients (BC:  $0.82 \pm 0.01$  versus HC:  $0.79 \pm 0.01$ ,  $p < 0.01$ ). Differences in post absorptive macronutrient oxidation rates were also observed when primary pathology was considered. The fasting rate of glucose oxidation was lower in HC patients when compared with control subjects ( $p < 0.01$ ) and when HC patients were compared with the BC group ( $p < 0.01$ ). The fasting rate of lipid oxidation was significantly higher than controls in both patient groups (controls versus BC,  $p < 0.01$ ; controls versus HC,

**FIGURE 5.2 SUBSTRATE OXIDATION IN CIRRHOTIC PATIENTS STRATIFIED BY DISEASE SEVERITY**



Mean ( $\pm$ SEM)

\* =  $p > 0.05$  versus Child's Pugh B

$p < 0.001$ ). The rate of protein oxidation was lower in HC patients when compared with controls ( $p < 0.05$ ) and BC patients ( $p < 0.05$ ) (Table 5.1).

### **OLT Patients**

Characteristics of the 23 patients who underwent OLT are shown in Table 3.6. No differences in post absorptive NPRQ or substrate oxidation rates were seen between OLT study time points (Table 5.2). Additionally, the influence of pre-OLT pathology (BC, HC) on fuel metabolism was not sustained after the first three months following OLT. When post-absorptive OLT fuel oxidation rates at 3 months were compared with control subjects, lipid oxidation rates were found to be higher in OLT patients ( $p < 0.05$ ). Compared with controls, no differences in lipid and glucose oxidation rates were seen 6 and 9 months after OLT. However, protein oxidation rates at 6 ( $p < 0.05$ ) and 9 ( $p < 0.05$ ) months post OLT were lower than control subjects.

## **Discussion**

### **Cirrhotic Patients**

The fasting metabolic profile of patients in the present study is characterised by enhanced lipid oxidation, a feature of liver cirrhosis that is well documented (Owen et al, 1983; Merli et al, 1990; Romijn et al, 1991; Muller et al, 1992). In the current study the rate of lipid oxidation in cirrhotic patients was found to be 25% greater and carbohydrate oxidation 30% less than fuel oxidation rates in control subjects. This finding is similar to the shift in fuel oxidation documented by other investigators

TABLE 5.1 FASTED SUBSTRATE OXIDATION RATES PER UNIT BODY MASS OF CONTROL SUBJECTS AND CIRRHOTIC PATIENTS

	Glucose (mg/min/kg BCM)	Lipid (mg/min/kg BCM)	Protein (mg/min/kg BCM)
Controls (n = 18)	4.67 (0.10)	1.55 (0.10)	1.47 (0.13)
Cirrhotic patients (n = 67)	3.86 <sup>a</sup> (0.21)	2.48 <sup>b</sup> (0.12)	1.17 (0.08)
Biliary cirrhosis (n = 35)	4.63 (0.32)	2.38 <sup>c</sup> (0.20)	1.37 (0.12)
Hepatocellular cirrhosis (n = 32)	3.15 <sup>c,d</sup> (0.23)	2.63 <sup>b</sup> (0.13)	0.95 <sup>a,e</sup> (0.10)

Mean (± SEM)

a = p<0.05 versus controls

b = p<0.001 versus controls

c = p<0.01 versus controls

d = p<0.01 versus biliary cirrhosis

e = p<0.05 versus biliary cirrhosis

TABLE 5.2 FASTING NPRQ AND SUBSTRATE OXIDATION RATES IN CONTROL SUBJECTS AND PATIENTS FOLLOWING LIVER TRANSPLANTATION

	NPRQ	Lipid oxidation mg/min/kg BCM	Glucose oxidation mg/min/kg BCM	Protein oxidation mg/min/kg BCM
OLT Patients (n = 23)				
- 3 months	0.83 (0.01)	2.13 (0.19) <sup>a</sup>	4.19 (0.42)	1.25 (0.10)
- 6 months	0.84 (0.01)	1.82 (0.17)	4.13 (0.43)	1.11 (0.08) <sup>a</sup>
- 9 months	0.84 (0.01)	1.88 (0.16)	4.10 (0.37)	1.17 (0.06) <sup>a</sup>
Controls (n = 18)	0.85 (0.01)	1.55 (0.10)	4.67 (0.10)	1.47 (0.13)
Mean (+ SEM)		a = p<0.05 versus controls		

(Eriksson et al, 1989; Green et al, 1991). Although in previous studies the fasting lipid oxidation rates tended to be higher than that found in the present study but the majority of research in this area has been performed in patients with alcohol related cirrhosis (Eriksson et al, 1989; Merli et al, 1990; Owen et al, 1993). Therefore, given the heterogeneity of the population in the current study, it seems unreasonable to assume that the variables that determine the rate and type of fuel oxidation should be the same in all cirrhotic patients regardless of the origin of primary pathology. Additionally, in the few studies that have included patients with cholestatic disease, no account was made for the influence of gender (Eriksson et al, 1989; Campillo et al, 1992). For example, fasting fuel utilisation rates as a unit of body weight may be higher in males because of their greater proportion of lean to fat mass. In the present study, cirrhotic patients were stratified by disease severity and primary diagnosis and all results expressed in relation to body cell mass to normalise fuel oxidation rates.

It has also been suggested that disease severity is associated with concomitant disturbances in energy and fuel metabolism (Muller et al, 1992). This was not apparent in the present study where no association between energy metabolism and disease severity was observed nor were there any differences in rates of lipid or glucose oxidation in relation to disease severity. However, the fasting rate of protein oxidation was found to be significantly lower in those patients classified as Child's Pugh C when compared with Child's Pugh B patients and this may be explained by the decrease in urinary nitrogen output seen in Child's Pugh C patients

(see Chapter 3). This finding may be indicative of reduced protein breakdown as evidenced by Neilsen et al (1995) who observed increased nitrogen retention in patients with liver cirrhosis. However, the anomaly that these differences arise between patients with Child's Pugh B and Child's Pugh C cirrhosis and not between patients with Child's Pugh A and C, is difficult to explain and cannot be accounted for by any aetiological bias.

The differences in fasting fuel utilisation observed when patients were stratified by primary aetiology, is an important yet hitherto unreported finding. As indicated by the NPRQ results, patients with HC cirrhosis exhibit a greater dependence on endogenous lipid fuel reserves when compared with controls or BC patients and this finding is confirmed when fasting substrate oxidation rates were calculated. Although, both patient groups exhibited enhanced post-absorptive lipid oxidation rates when compared with controls these were highest in HC patients. In addition, only HC patients exhibited a depressed post absorptive rate of glucose oxidation when compared with both control subjects and BC patients.

These results suggest that BC patients have some remaining capacity for glycogen storage following an overnight fast but this is not apparent in HC patients. This finding is supported by Green et al (1991) who in a study of only seven BC patients and healthy controls observed an increase in post-absorptive lipid oxidation rates but no differences in glucose and protein oxidation. Furthermore, no differences in total free fatty acids in BC patients were observed whereas in studies in HC

patients, elevations in fasting free fatty acids and glycerol have been reported (Owen et al, 1983; Meril et al, 1985; Romijn 1991) and suggests the rate of lypolysis in BC disease may be limited. The only other study which stratified for primary aetiology was conducted by Muller and colleagues(1992) who noted no differences in fasting substrate oxidation rates between patients with BC, alcoholic liver disease and hepatic cirrhosis. However, it is worth noting that although not reaching statistical significance the rate of carbohydrate oxidation was highest and lipid oxidation lowest in the BC group.

In conclusion, the fasting metabolic profile of patients with HC cirrhosis is indicative of a longer period of fasting. This current study on post-absorptive substrate oxidation rates in cirrhotic patients has highlighted important metabolic differences between patients with BC and HC cirrhosis and suggests re-evaluation of previous generalisations on fuel utilisation in chronic liver disease.

### **OLT Patients**

This is one of the first longitudinal metabolic studies on patients subjected to liver transplantation. It is not unreasonable to suggest that following OLT, the fasting profile of oxidative metabolism will normalise. In the present study, three months after OLT neither disease severity or primary pathology had any influence on substrate oxidation rates. Whilst no differences in NPRQ or fuel oxidation rates between OLT study time points were apparent, the fasting lipid oxidation rate was significantly higher in OLT recipients three months after transplantation when



compared with controls. This finding could be attributed to the continuing consequences of the surgical trauma of the OLT procedure. This view is supported by the oxidation rates for lipid and glucose which were comparable to control values at six and nine months after transplantation.

Interestingly, six and nine months following OLT protein oxidation rates decreased between patients and controls. No difference in protein oxidation rates patients three months after OLT and controls was observed. It could be suggested that as a result of OLT and subsequent lack of co-ordinated metabolic regulation, there may be a decrease in protein turnover analogous to that of starvation (Rennie, 1995). Therefore the loss of hepatic afferent signalling relaying messages to the hypothalamus may be interpreted as reduced nutrient availability (Niiijima, 1982) and may also be reflected in a fall in urinary nitrogen excretion (Frayn, 1997).

In summary, the post-absorptive availability of the major energy providing macronutrients is comparable to control subjects from three months OLT. There is, after all, no physiological reason why OLT patients oxidative processes would in the long term be different from normal.

## CHAPTER 6

### SUBSTRATE UTILISATION IN RESPONSE TO FEEDING IN LIVER CIRRHOSIS AND LIVER TRANSPLANT RECIPIENTS

The observations on nutrient metabolism in fasted cirrhotic patients are interesting but limiting in that they give a static view of substrate oxidation. Clearly metabolism is a complex and dynamic process involving a system of substrate flow and integration. Metabolic information derived from neural and hormonal signalling will be relayed to and integrated in the brain or to be more precise in the hypothalamus, the ultimate controller of energy homeostasis. In response to the availability of endogenous substrates the brain acts to regulate both short and longterm energy intake and expenditure. Given the hypothesis that altered fuel metabolism in liver cirrhosis could influence nutrient intake, it is important to examine the integrated response to feeding.

To date substrate utilisation in either the fasted or fed state has not been examined in OLT patients.

#### **Patients and Methods**

##### **Patients**

Thirty-six patients with chronic liver disease were entered into the feeding limb of the study. Eighteen patients were submitted to OLT and had an uncomplicated recovery and were entered into the feeding limb of the study. These OLT patients were reviewed on a three monthly basis on

three occasions. Eighteen healthy volunteers were also recruited. Patients and controls are fully described in Chapter 4.

## **Methods**

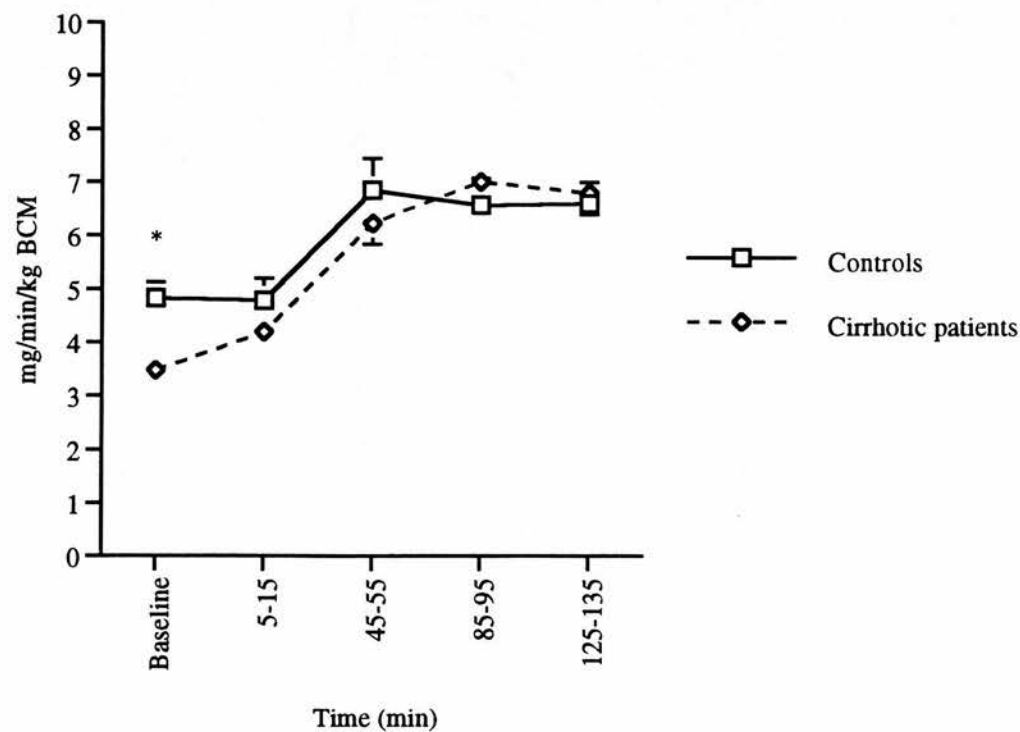
Following determination of post absorptive energy expenditure and substrate oxidation rates. All subjects were prescribed a liquid meal (15kJ/kg body weight) and required to consume the meal within five minutes (time 0). Indirect calorimetry was performed at five, 45, 85 and 125 minutes after meal ingestion. In addition a six hour urine collection was taken for urinary nitrogen determination. Results of indirect calorimetry and urinary nitrogen were used in the calculation of substrate oxidation in the post ingestive period. Methods are described in detail in Chapter 2.

## **Results**

### **Cirrhotic Patients**

Characteristics of the cirrhotic patients and control subjects is given in Table 4.1. Glucose and lipid oxidation rates in all control subjects and cirrhotic patients are shown in Figures 6.1 and 6.2. When compared with controls the fasting rate of glucose oxidation was lower in cirrhotic patients (controls:  $4.80 \pm 0.30$  mg/min/kg BCM versus cirrhotics:  $3.40 \pm 0.23$  mg/min/kg BCM,  $p < 0.001$ ). In response to feeding, no differences in glucose oxidation was observed between controls and the cirrhotic patients. The peak response was seen 45 minutes postprandially in the control group. Fasting lipid oxidation rates were significantly elevated in

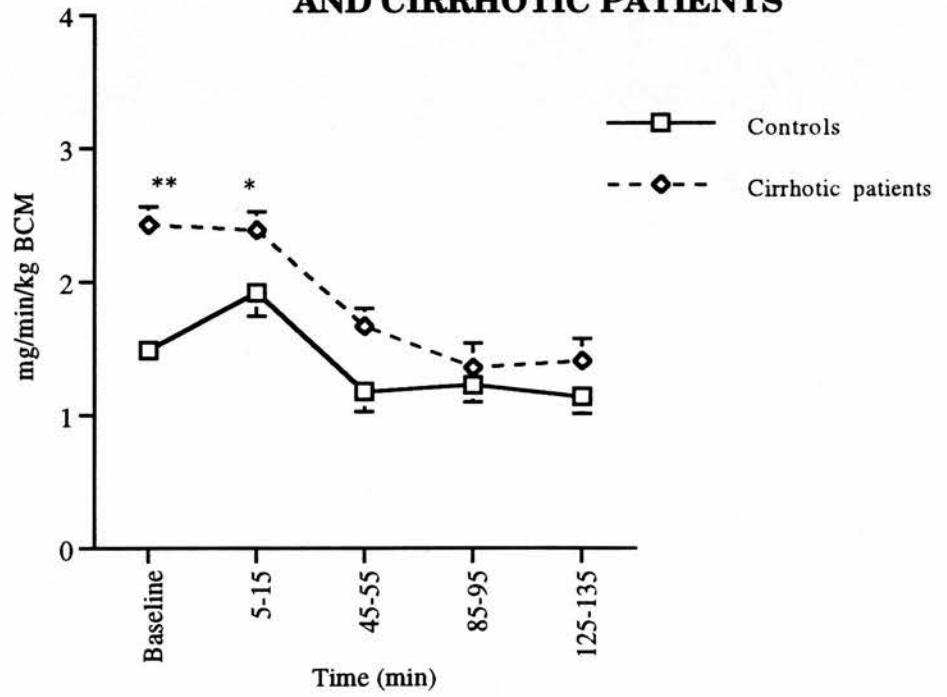
**FIGURE 6.1 GLUCOSE OXIDATION IN CONTROLS AND CIRRHOTIC PATIENTS**



Mean ( $\pm$ SEM)

\* =  $p < 0.001$  versus controls

**FIGURE 6.2 LIPID OXIDATION IN CONTROLS AND CIRRHOTIC PATIENTS**



Mean ( $\pm$ SEM)

\* =  $p < 0.05$  versus controls

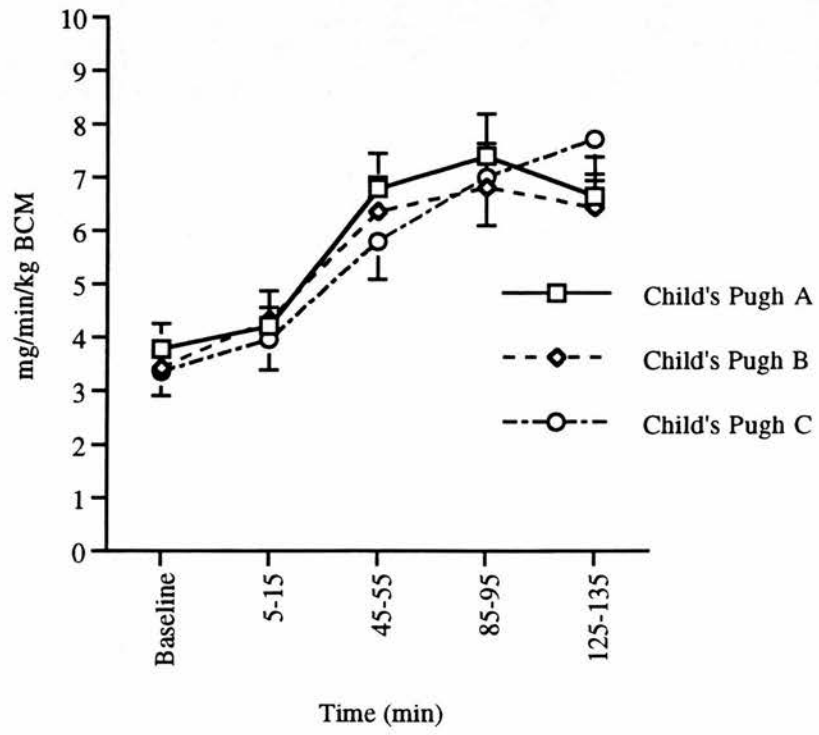
\*\* =  $p < 0.0001$  versus controls

cirrhotic patients when compared with control subjects (controls:  $1.49 \pm 0.11$  mg/min/kg BCM versus cirrhotics:  $2.34 \pm 0.13$  mg/min/kg,  $p < 0.0001$ ) and at the first post-prandial time point (controls:  $1.92 \pm 0.17$  mg/min/kg BCM versus cirrhotics:  $2.39 \pm 0.14$  mg/min/kg BCM,  $p < 0.05$ ). Protein oxidation rate was lower in cirrhotic patients when compared to control subjects (controls:  $1.52 \pm 0.15$  mg/min/kg BCM versus cirrhotics:  $1.12 \pm 0.11$ ,  $p < 0.05$ ).

When patients were classified by the modified Child's Pugh score, no differences in glucose and lipid oxidation rates were observed in the fasting state or in response to the test meal (Figures 6.3, 6.4). The peak glucose responses were at 85, 85 and 125 minutes in Child's Pugh A, B and C patients respectively. However, when patients were stratified by primary aetiology, those with HC cirrhosis had depressed rates of glucose oxidation in the fasted state when compared with controls and BC patients (Glucose: controls:  $4.83 \pm 0.30$  mg/min/kg BCM versus HC:  $2.89 \pm 0.28$  mg/min/kg BCM,  $p < 0.001$ ; HC versus BC:  $4.03 \pm 0.31$  mg/min/kg BCM,  $p < 0.05$ ) and at the first post-prandial time point (Time point 1: Glucose: controls  $4.79 \pm 0.42$  mg/min/kg BCM versus HC:  $3.30 \pm 0.35$  mg/min/kg BCM,  $p < 0.05$ ; HC versus BC:  $4.84 \pm 0.37$  mg/min/kg BCM,  $p < 0.05$ ) (Figure 6.5).

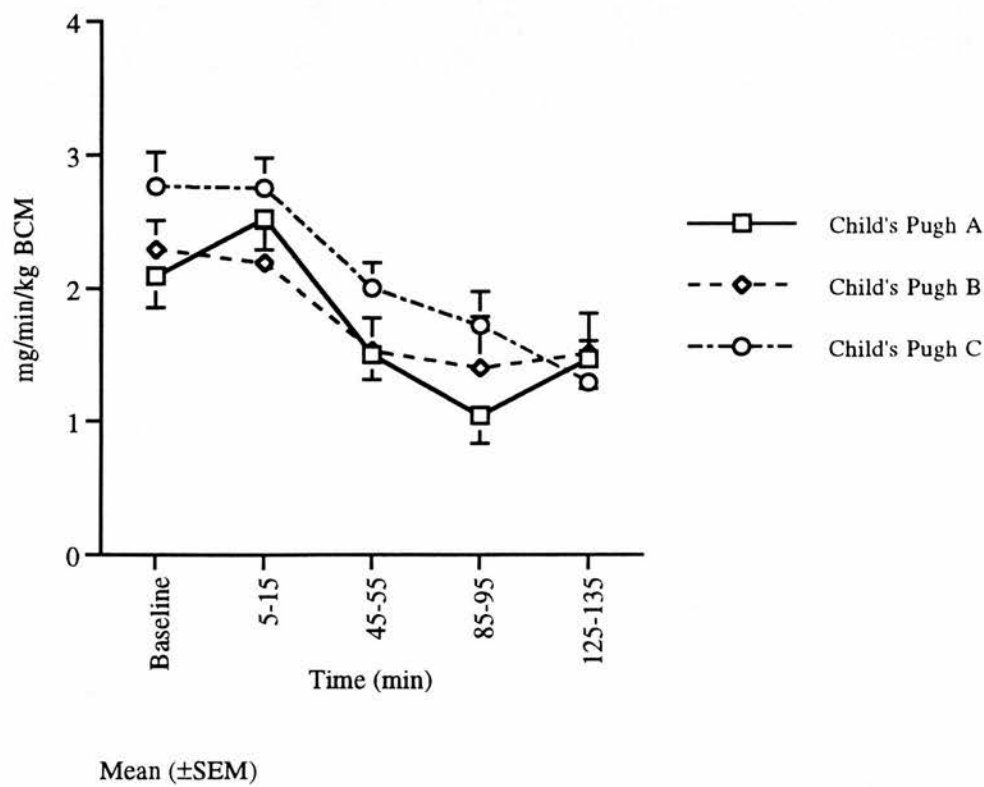
Complementary to this reduction in glucose oxidation, HC patients exhibited significantly elevated rates of lipid oxidation in the fasting state when compared with control subjects and BC patients (controls:  $1.49 \pm 0.11$  mg/min/kg BCM versus HC:  $2.75 \pm 1.81$  mg/min/kg BCM,  $p < 0.0001$ ; HC versus BC:  $1.95 \pm 0.15$  mg/min/kg BCM,  $p < 0.01$ ).

**FIGURE 6.3 GLUCOSE OXIDATION IN PATIENTS  
STRATIFIED BY DISEASE SEVERITY**



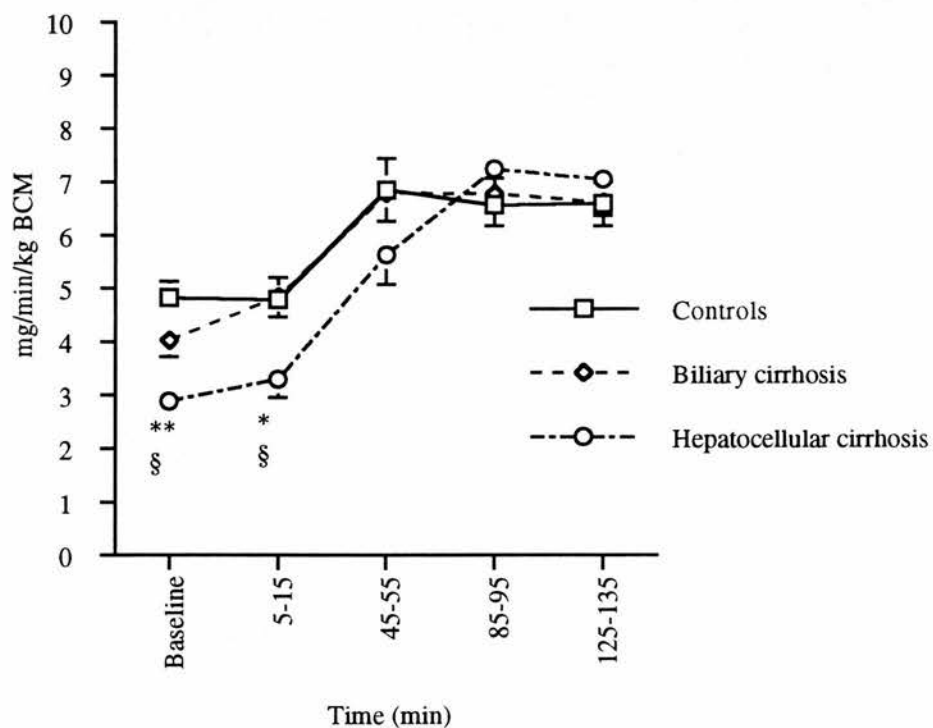
Mean ( $\pm$ SEM)

**FIGURE 6.4 LIPID OXIDATION IN PATIENTS STRATIFIED BY DISEASE SEVERITY**





**FIGURE 6.5 GLUCOSE OXIDATION IN PATIENTS WITH BILIARY AND HEPATOCELLULAR CIRRHOSIS**



Mean ( $\pm$ SEM)

\* =  $p < 0.05$  versus controls

\*\* =  $p < 0.001$  versus controls

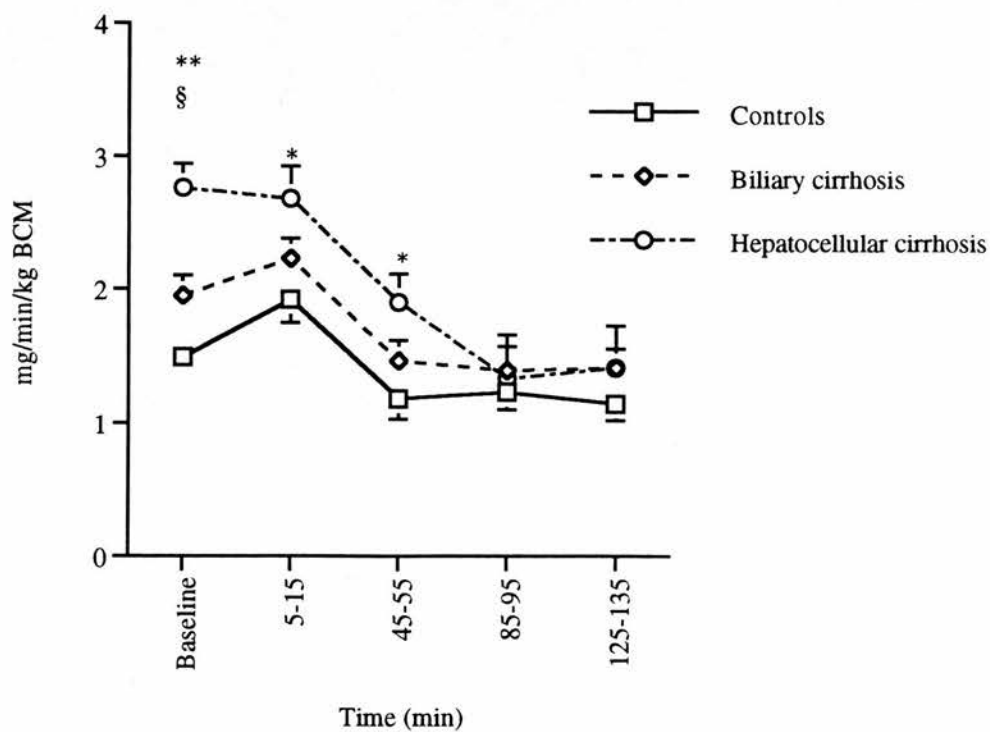
§ =  $p < 0.05$  versus biliary cirrhosis

Additionally, the HC patients demonstrated increased rates of lipid oxidation at 5 (controls:  $1.89 \pm 0.15$  mg/min/kg BCM versus HC:  $2.68 \pm 0.24$  mg/min/kg BCM,  $p < 0.05$ ) and 45 (controls:  $1.18 \pm 0.15$  mg/min/kg BCM versus HC:  $1.90 \pm 0.21$  mg/min/kg BCM,  $p < 0.05$ ) minutes after meal ingestion (Figure 6.6). The change in glucose and lipid oxidation rates in response to feeding are shown in Figure 6.7. The HC group showed an early and rapid switch to glucose oxidation when compared with control subjects (controls:  $43 \pm 12.9\%$  compared with HC:  $184 \pm 44.6\%$ ;  $< 0.001$ ) and patients with BC (HC compared with BC:  $79 \pm 12.7\%$ ;  $p < 0.05$ ). Hepatocellular cirrhotic patients exhibited significantly lower protein oxidation rates in response to feeding when compared with controls (controls:  $1.50 \pm 0.15$  mg/min/kg BCM versus HC:  $0.93$  mg/min/kg BCM,  $p < 0.05$ ). Protein oxidation rates were similar in BC and control subjects during the test meal study period.

### OLT Patients

Seven male and 11 female patients (mean age  $52.8 \pm 2.4$  years) were studied following OLT. All ten BC patients were female and seven of the eight HC patients were male. Non-protein respiratory quotient, glucose and lipid oxidation rates in OLT patients and control subjects are shown in Table 6.1. The fasting profile of pre-OLT patients, when compared with controls, was similar to that described in the preceding Chapter.

**FIGURE 6.6 LIPID OXIDATION IN PATIENTS WITH BILIARY AND HEPATOCELLULAR CIRRHOSIS**



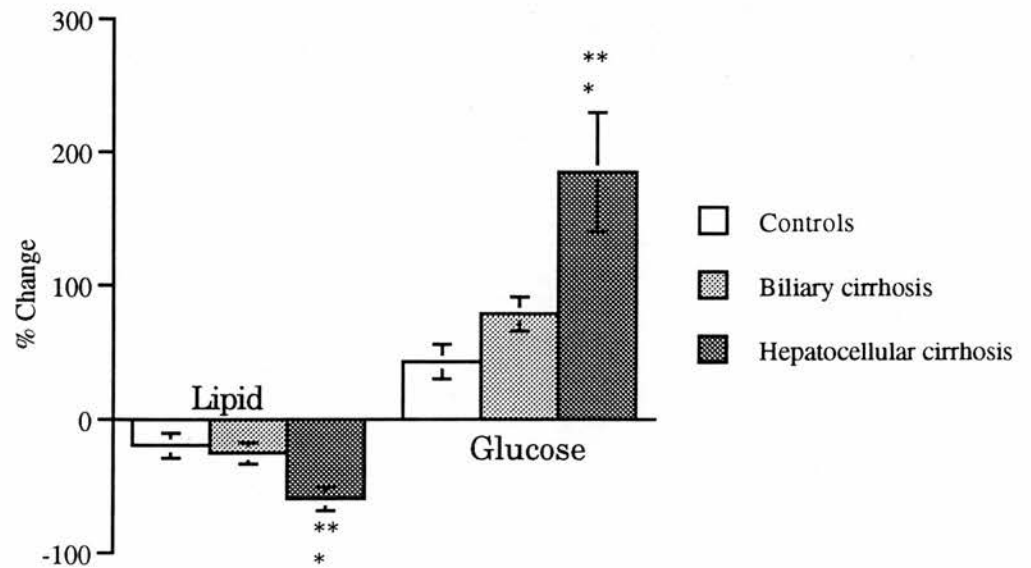
Mean ( $\pm$ SEM)

\* =  $p < 0.05$  versus controls

\*\* =  $p < 0.0001$  versus controls

§ =  $p < 0.01$  versus controls

**FIGURE 6.7 CHANGE IN LIPID AND GLUCOSE OXIDATION RATES IN PATIENTS WITH BILIARY AND HEPATOCELLULAR CIRRHOSIS**



Mean ( $\pm$ SEM)

\*\* =  $p < 0.001$  versus controls

\* =  $p < 0.05$  versus biliary cirrhosis

**TABLE 6.1 NON-PROTEIN RESPIRATORY QUOTIENT, GLUCOSE  
AND LIPID OXIDATION RATES IN RESPONSE TO FEEDING  
FOLLOWING LIVER TRANSPLANTATION**

	NPRQ	Glucose (mg/min/kg BCM)	Lipid
Pre-OLT Baseline	0.81 (0.01) <sup>b</sup>	3.8 (0.25) <sup>c</sup>	2.4 (0.18) <sup>a</sup>
5-15 min	0.81 (0.01)	4.2 (0.39)	2.7 (0.18) <sup>d</sup>
45-55 min	0.88 (0.01)	7.2 (0.46)	1.5 (0.18)
85-95 min	0.90 (0.01)	7.8 (0.59) <sup>e</sup>	1.3 (0.20)
125-135 min	0.91 (0.01)	7.6 (0.60)	1.2 (0.22)
3 month OLT Baseline	0.83 (0.01)	4.4 (0.56)	2.2 (0.21)
5-15 min	0.83 (0.02)	5.3 (0.73)	2.3 (0.24)
45-55 min	0.89 (0.01)	7.3 (0.89)	1.6 (0.28)
85-95 min	0.88 (0.01)	7.1 (0.69)	1.7 (0.18) <sup>f</sup>
125-135 min	0.89 (0.01)	8.3 (0.87)	1.4 (0.18)
6 month OLT Baseline	0.83 (0.01)	4.2 (0.44)	2.0 (0.18)
5-15 min	0.85 (0.01)	5.1 (0.50)	2.0 (0.18)
45-55 min	0.89 (0.01)	6.6 (0.53)	1.4 (0.17)
85-95 min	0.91 (0.01)	6.8 (0.45)	1.1 (0.14)
125-135 min	0.92 (0.01)	7.6 (0.33)	1.0 (0.12)
9 month OLT Baseline	0.83 (0.01)	4.1 (0.42)	2.1 (0.19)
5-15 min	0.82 (0.01)	4.4 (0.49)	2.3 (0.20)
45-55 min	0.88 (0.02)	6.2 (0.58)	1.6 (0.22)
85-95 min	0.89 (0.01)	6.6 (0.47)	1.6 (0.22)
125-135 min	0.90 (0.01)	7.0 (0.53)	1.4 (0.21)
Control-Baseline	0.85 (0.01)	4.8 (0.30)	1.9 (0.10)
5-15 min	0.83 (0.01)	4.9 (0.37)	1.6 (0.30)
45-55 min	0.89 (0.01)	6.9 (0.59)	1.2 (0.15)
85-95 min	0.89 (0.01)	6.9 (0.50)	1.2 (0.13)
125-135 min	0.89 (0.01)	6.7 (0.40)	1.1 (0.12)

Mean ( $\pm$  SEM)

a =  $p < 0.001$  versus controls

b =  $p < 0.01$  versus controls

c =  $p < 0.05$  versus controls

d =  $p < 0.01$  versus 6 months OLT

e =  $p < 0.05$  versus 9 months OLT

f =  $p < 0.05$  versus 6 months OLT

## Overview

No differences in lipid and glucose oxidation rates were seen between control subjects and patients three, six and nine months after OLT. During the observation period, protein oxidation was significantly lower three and six months OLT when compared to the control group (Controls:  $1.52 \pm 0.14$  mg/min/kg BCM versus 3 month OLT:  $1.26 \pm 0.10$  mg/min/kg BCM;  $p < 0.05$  : Controls versus 6 month OLT:  $1.11 \pm 0.80$  mg/min/kg BCM;  $p < 0.05$ ).

## Six Months

At six months following OLT patients exhibited a significantly lower lipid oxidation rate five to 15 minutes after meal ingestion when compared with pre-OLT values. In addition by six months OLT, lipid oxidation rates were significantly lower 85-95 minutes after meal ingestion when compared to the same post ingestive time point three months after transplantation.

## Nine Months

Nine months following OLT, a significant decrease in glucose oxidation rate was observed only at the 85-95 min post-prandial study time point when compared with the same time point before transplantation (Table 6.1). During the post-meal observation period, no differences in protein oxidation rates between patient groups were observed.

## Discussion

### Cirrhotic Patients

The current study is one of the few that has examined substrate flux from the fasting to the fed state in cirrhotic patients. This study has allowed stratification of patients by disease severity and primary aetiology.

The response to feeding observed in cirrhotic patients suggests there is an early switch to glucose oxidation and this is mirrored by a depression of lipid oxidation rates. Campillo and colleagues (1992), in a study of only ten patients with alcoholic cirrhosis also observed this early switch to glucose oxidation but the magnitude of change appeared much greater than that found in the current study. There were differences in experimental design in Campillo's work and the current study. The test meal employed by Campillo et al (1992) was much larger (63 kJ/kg) and the period of investigation was twice that of the present study (6 hours compared with 3 hours). Furthermore, the study by Campillo et al (1992) was only in HC disease whereas in the present study there were an equal number of HC and BC patients.

The finding that disease severity did not elicit a clear metabolic demarcation in this stable patient group is not surprising given the arbitrary nature of the Child's Pugh scoring system. Differences between patient groups and control subjects and within patient groups were observed when patients were stratified by primary aetiology. Interestingly no differences were observed between the BC group and controls in response to feeding. The only other comparable study (Green

et al, 1991) to examine the response to feeding looked at seven BC patients and they observed no differences in substrate metabolism between patients and controls in the basal state or four hours following meal ingestion. In this study, substrate utilisation was only measured at two time points, nevertheless the work of Green and colleagues' (1991) does support the findings of the current investigation where the response to feeding in BC patients was similar to controls.

In the present study HC cirrhotic patients exhibited differences in fuel utilisation in both the fasting and fed state when compared with controls and BC patients. Following ingestion of the meal HC patients showed an early and rapid switch to glucose oxidation when compared with control subjects and BC patients. In response to the increase in glucose oxidation rate there was a concomitant decrease in lipid oxidation rate. These observed differences between BC and HC patients in response to feeding may have important implications in the prescribing of nutritional regimens since alterations in metabolism could influence ingestive behaviour. It may be postulated that in HC patients, the rapid and early switch to glucose oxidation could, through hepatic neural signalling, reduce satiety thresholds bringing about early meal termination. This phenomena could contribute to the poor spontaneous oral intake found in patients with cirrhosis (Cabre et al, 1990).

In the present study the shift from lipid to glucose oxidation highlights differences between patients with cholestatic and hepatocellular cirrhosis. It has been suggested that the avid utilisation of ingested carbohydrate is an adaptive response to the reduced synthetic storage



capacity of hepatocytes and a reduction in the activity of the enzyme glycogen synthetase (Kruszynska et al, 1988). It would seem likely that given this rapid increase in glucose oxidation there may be a significant and uncontrolled elevation in blood glucose but hyperglycaemia in cirrhosis is generally avoided. For example, Eriksson et al, 1991 has shown that following ingestion of a carbohydrate rich meal, circulating insulin concentrations increase. This may facilitate maximal glucose oxidation in patients who have defective storage mechanisms (Petrides and De Fronzo, 1989). The decrease in lipid oxidation rates will facilitate storage and availability for utilisation in the post absorptive period.

This study has identified hitherto unreported metabolic differences to feeding in cirrhotic patients with different primary pathologies. This finding may have important implications in prescribing nutritional support for cirrhotic patients where high carbohydrate regimens may indirectly act to induce early satiety and are perhaps inappropriate for patients with HC cirrhosis.

### **OLT Patients**

Following OLT, the rates of glucose and lipid oxidation were comparable to the control group. This finding is not unexpected given that the glycogen storage capacity in uncomplicated OLT patients should be normal. The recipients healthy hepatocytes are, of course, now able to designate macronutrients delivered by the portal vein to be synthesised, stored or oxidised.

The depressed rate of protein oxidation found in patients at three and six months after transplantation compared with controls cannot be easily explained. It could be associated with hepatic denervation where central processors may interpret the lack of hepatic neural signalling as nutrient deprivation. In addition immunosuppression may reduce protein turnover rates. In the present study, neither of these possibilities that may account for the reduced rate of protein oxidation found in patients six and nine following OLT can be confirmed.

The only other study that has examined fuel utilisation in response to feeding among OLT patients was by Green and colleagues (1991). This group observed no differences in fuel oxidation rates between seven patients and seven control subjects. In order to allow examination of the progressive impact of meal ingestion on lipid and glucose oxidation, the present study determined calculated rates of oxidation on four occasions over a two hour postprandial period. This methodological approach was considered important as the postprandial profile of substrate utilisation may be implicated in the disproportionate increase in fat mass observed in OLT patients (Chapter 3). Glycogen storage capacity is limited and when replete the rate of glucose oxidation will increase concomitantly with intake thereby suppressing mobilisation and oxidation of lipid and lead to expansion of body fat stores (Tremblay et al, 1991; Thomas et al, 1992).

There was a tendency for NPRQ to increase during the later postprandial period at six months OLT when compared with controls. Whilst the difference in NPRQ failed to reach significance it does suggest a tendency for the six month OLT patients to oxidise glucose relative to

lipid. When pre-OLT patient values were compared to the nine month OLT group, a decrease in glucose oxidation was seen but only at the 85-95 min study time point ( $p<0.05$ ). This may in part be explained by the transient elevation in glucose oxidation seen in cirrhotic patients.

In the present study there is no substantive evidence to suggest that following OLT, qualitative differences in fuel metabolism could contribute to the increase in fat mass seen in this OLT population. However, it is possible that they may be masked by the composition of the test meal prescribed, that is the meal given may have been insufficient both in terms of energy density and composition (Raben et al, 1994; Larson et al, 1995). For example, Astrup et al (1994), who examined the effect of diet on RQ, only found an increase in lipid oxidation rates on a diet that comprised 50% of the energy derived from fat. Ingestion of large amounts of fat promotes fat oxidation but only to a limited extent (Flatt, 1995). The prescription of the test meal in the current study was dictated by the acceptability of the meal to the cirrhotic population.

The findings of the present study do not preclude the possibility that weight gain following OLT may be associated with a reduced capacity to raise fat oxidation in parallel with moderate to high fat intakes and would promote fat storage. Currently, the understanding of the mechanisms involved in weight gain following liver transplantation is limited but further studies that focus on the use of high fat/energy dense meal challenges are required to confirm or refute any association between fuel utilisation and fat storage.

## CHAPTER 7

### INSULIN AND GLUCOSE RESPONSE TO FEEDING IN LIVER CIRRHOSIS AND LIVER TRANSPLANT RECIPIENTS

The presence of glucose intolerance in liver cirrhosis has been recognised for sometime (Berkowitz, 1969) but the mechanisms involved in defective insulin mediated glucose metabolism have not been attributed to any single factor (Nolte et al, 1995). It has been suggested that insulin resistance resulting in defective glucose disposal is implicated in the glucose intolerance reported in liver cirrhosis (Petrides et al, 1994). The presence of portasystemic shunting may cause diminished hepatic insulin and glucose clearance (Bosch et al, 1984) and other investigators suggest that defects in peripheral insulin receptor/post-receptor function also plays a significant role in glucose intolerance (Cavallo-Perin et al, 1985; Kruszynska and McIntyre, 1991). There is, however, no consensus as to the exact mechanisms involved in the hyperglycaemia/hyperinsulinaemia of cirrhosis. However, what is clear is that in cirrhosis glucose storage capacity (i.e. hepatic and muscle glycogen formation) is significantly reduced and this may increase circulating glucose concentrations.

Following solid organ transplantation, a proportion of patients develop post-transplant hyperglycaemia (Roth et al, 1989; Tabasco-Minguillan et al, 1993). The aetiology of new onset DM after transplantation is considered multifactorial but the diabetogenic effects of

the immunosuppressive reagents are thought to play a major role (Tabasco-Minguillan et al, 1993).

In Chapters 5 and 6 the substrate utilisation in the fasted and fed state was examined in cirrhotic patients and control, subjects. This Chapter focuses on the insulin and glucose profiles under basal conditions and in response to feeding in stable cirrhotic patients and a group of healthy controls. A sub-group of patients with liver cirrhosis were submitted to OLT and were reviewed three, six and nine months after transplantation.

## **Patients and Methods**

### **Patients**

Thirty-six patients with chronic liver disease were entered into the feeding limb of the study. Eighteen patients were submitted to OLT, had an uncomplicated recovery and were entered into the feeding limb of the study. These OLT patients were reviewed on a three monthly basis on three occasions. Eighteen healthy volunteers were also recruited. Patients and controls are fully described in Chapter 4.

### **Methods**

Blood samples were taken for determination of plasma glucose concentration at baseline and every 20 minutes for two hours following ingestion of the test meal. Plasma insulin concentration was determined at baseline, 60 and 120 minutes (see Figure 2.1). The integrated glucose

and insulin and glucose response to the test meal was calculated using the trapezoid method. Methods are fully described in Chapter 2.

## Results

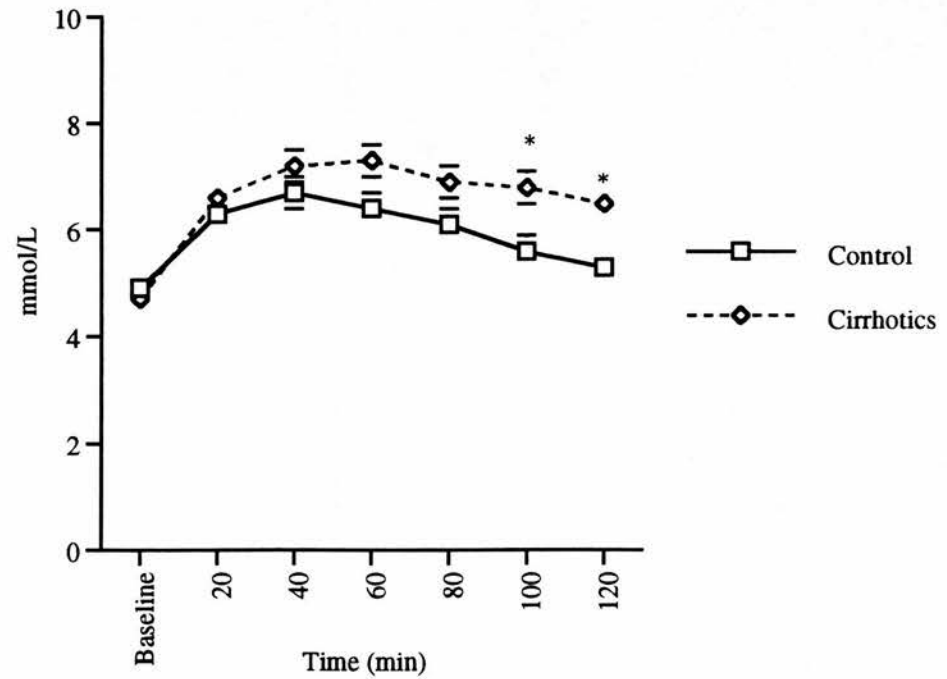
### Cirrhotic Patients

Characteristics of the cirrhotic patient population and control group are shown in Table 4.1. Basal plasma glucose concentrations and the two hour response to a mixed meal in cirrhotic patients and controls is shown in Figure 7.1 and 7.2. Whilst there were no significant differences in fasting blood glucose concentrations between cirrhotic patients and controls, plasma glucose values were significantly elevated in cirrhotic patients at the 100 and 120 minute post-prandial time point (100 min: controls  $5.6 \pm 0.24$  mmol/l compared to cirrhotics  $6.8 \pm 0.30$  mmol/l,  $p < 0.05$ ; 120min: controls  $5.3 \pm 0.28$  mmol/l compared to cirrhotics  $6.5 \pm 0.23$  mmol/l;  $p < 0.05$ ).

In addition, when compared with controls, cirrhotic patient's fasting plasma insulin concentrations were significantly higher (basal: controls  $5.8 \pm 0.62$  mU/l compared to cirrhotics  $13.5 \pm 1.8$  mU/l,  $p < 0.01$ ). Insulin concentrations remained significantly elevated one and two hours after meal ingestion (60 min: controls  $27.9 \pm 2.9$  mU/l compared to cirrhotics  $64.4 \pm 12.5$  mU/l,  $p < 0.05$ ; 120 min: controls  $13.9 \pm 1.9$  mU/l compared to  $54.4 \pm 12.2$  mU/l,  $p < 0.05$ ) (Figure 7.2).

When patients were classified by Child's Pugh modified score no differences in glucose (Figure 7.3) or insulin concentration (Figure 7.4) was apparent in either the fasted or fed state. Patients were also sub-divided

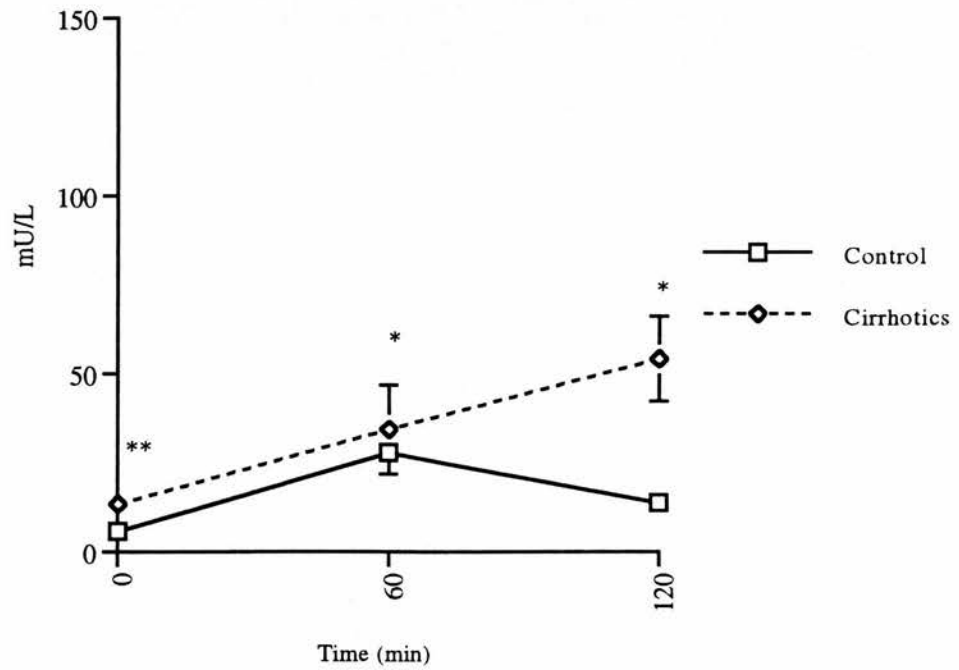
**FIGURE 7.1 GLUCOSE RESPONSE TO FEEDING IN CONTROLS AND CIRRHOTIC PATIENTS**



Mean ( $\pm$ SEM)

\* =  $p < 0.05$  versus controls

**FIGURE 7.2 INSULIN RESPONSE TO FEEDING IN CONROLS AND CIRRHOTIC PATIENTS**



mean ( $\pm$ SEM)

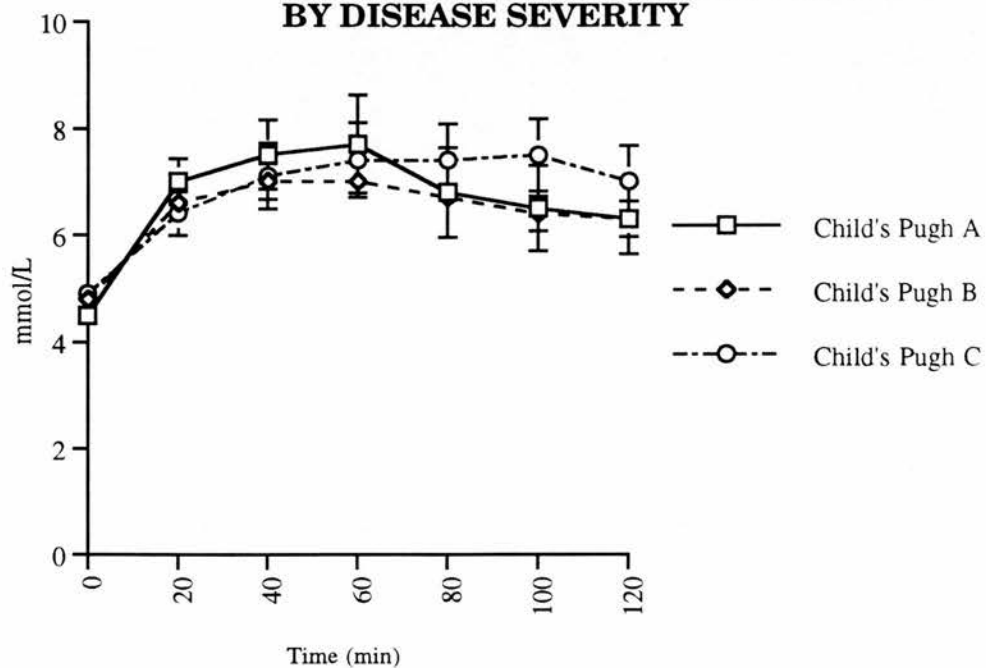
\* =  $p < 0.05$  versus controls

\*\* =  $p < 0.01$  versus controls

AUC =  $p < 0.05$  versus controls

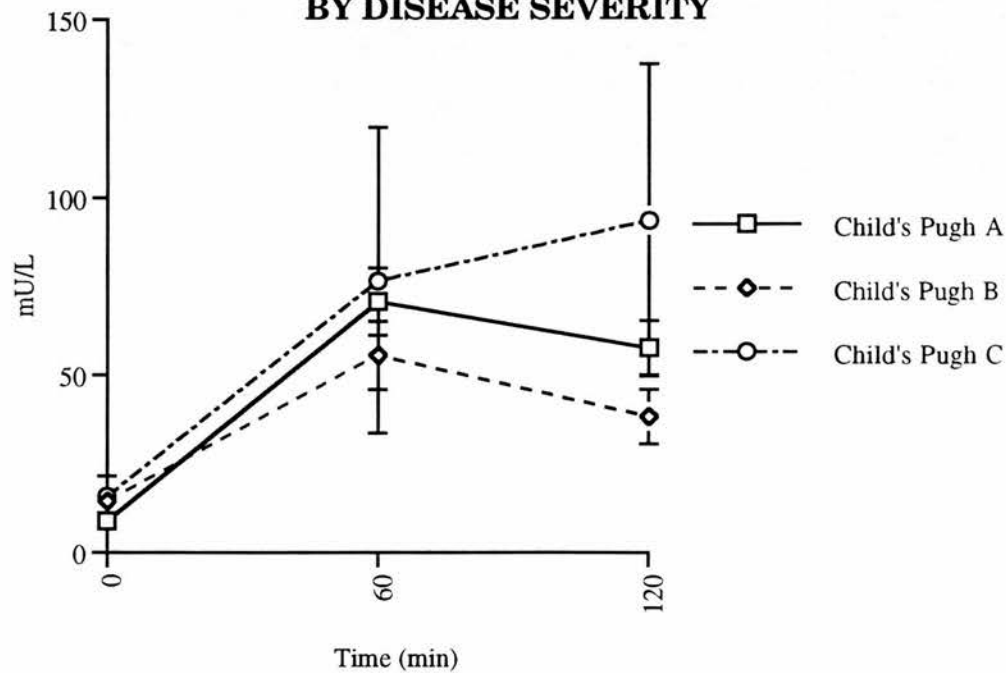


**FIGURE 7.3 GLUCOSE RESPONSE TO FEEDING IN CIRRHOTIC PATIENTS STRATIFIED BY DISEASE SEVERITY**



mean ( $\pm$ SEM)

**FIGURE 7.4 INSULIN RESPONSE TO FEEDING IN  
CIRRHOTIC PATIENTS STRATIFIED  
BY DISEASE SEVERITY**

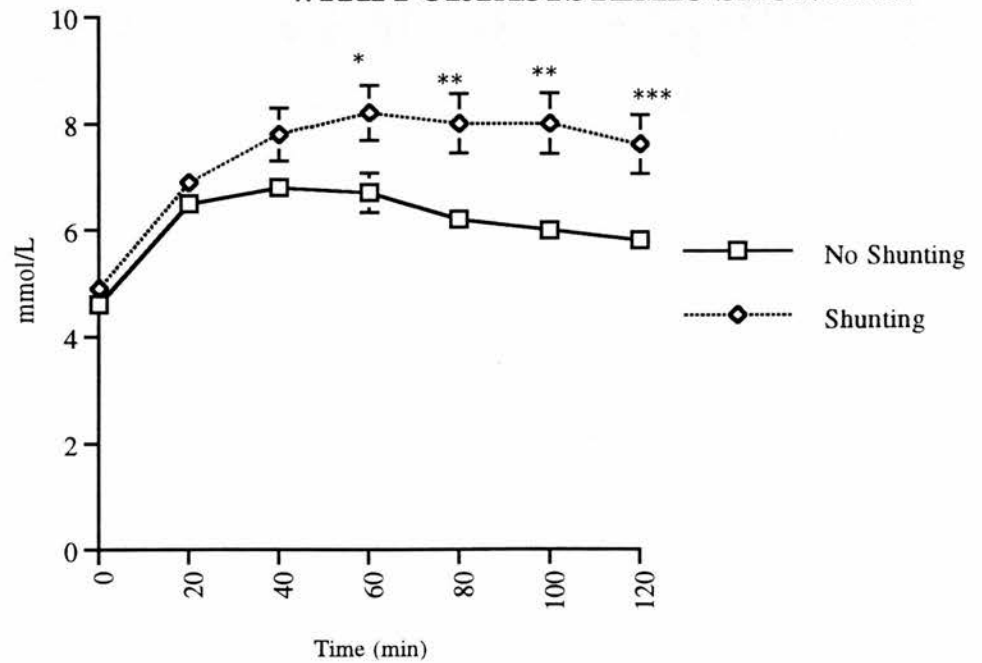


by the presence or not of portal systemic shunting evidenced by the presence of varices (shunting n = 14; no shunting n = 22). Those patients with evidence of shunting had significantly higher blood glucose values than the control group at 60 through to 120 minutes post-prandially (60 min: no shunting  $6.7 \pm 0.31$  mmol/l compared to shunting  $8.2 \pm 0.52$  mmol/l;  $p < 0.05$ ; 80 min: no shunting  $6.2 \pm 0.27$  mmol/l compared to shunting  $8.0 \pm 0.56$  mmol/l;  $p < 0.01$ ; 100 min: no shunting  $6.0 \pm 0.24$  mmol/l compared to shunting  $8.1 \pm 0.58$  mmol/l,  $p < 0.01$ ; 120 min: no shunting  $5.7 \pm 0.30$  mmol/l compared to  $7.6 \pm 0.55$  mmol/l,  $p < 0.01$ ) (Figure 7.5). No differences in the basal or post-prandial insulin response was observed in patients with or without portal hypertension (Figure 7.6).

Patients were also classified by primary aetiology and plasma glucose concentration was higher in HC patients than controls at 80, 100 and 120 minutes post-prandially (80 min: controls  $6.1 \pm 0.25$  mmol/l compared to HC  $7.5 \pm 0.38$  mmol/l,  $p < 0.05$ ; 100 min: controls  $5.6 \pm 0.24$  mmol/l compared to HC  $7.4 \pm 0.38$  mmol/l,  $p < 0.01$ ; 120 min: controls  $5.3 \pm 0.23$  mmol/l compared to HC  $6.9 \pm 0.36$  mmol/l,  $p < 0.01$ ) (Figure 7.7). In addition, 80 minutes after meal ingestion differences in blood glucose concentrations were seen between patients with cholestatic and hepatocellular cirrhosis (80 min: BC  $6.2 \pm 1.7$  mmol/l compared to HC  $7.5 \pm 1.6$  mmol/l;  $p < 0.05$ ).

Insulin profiles were also significantly different when patients were stratified by primary aetiology (Figure 7.8). The HC patients had significantly elevated basal plasma insulin concentrations when compared to control subjects or BC patients (controls:  $5.8 \pm 0.62$  mU/l compared

**FIGURE 7.5 GLUCOSE RESPONSE TO FEEDING IN CIRRHOTIC PATIENTS WITHOUT AND WITH PORTASYSTEMIC SHUNTING**



mean ( $\pm$ SEM)

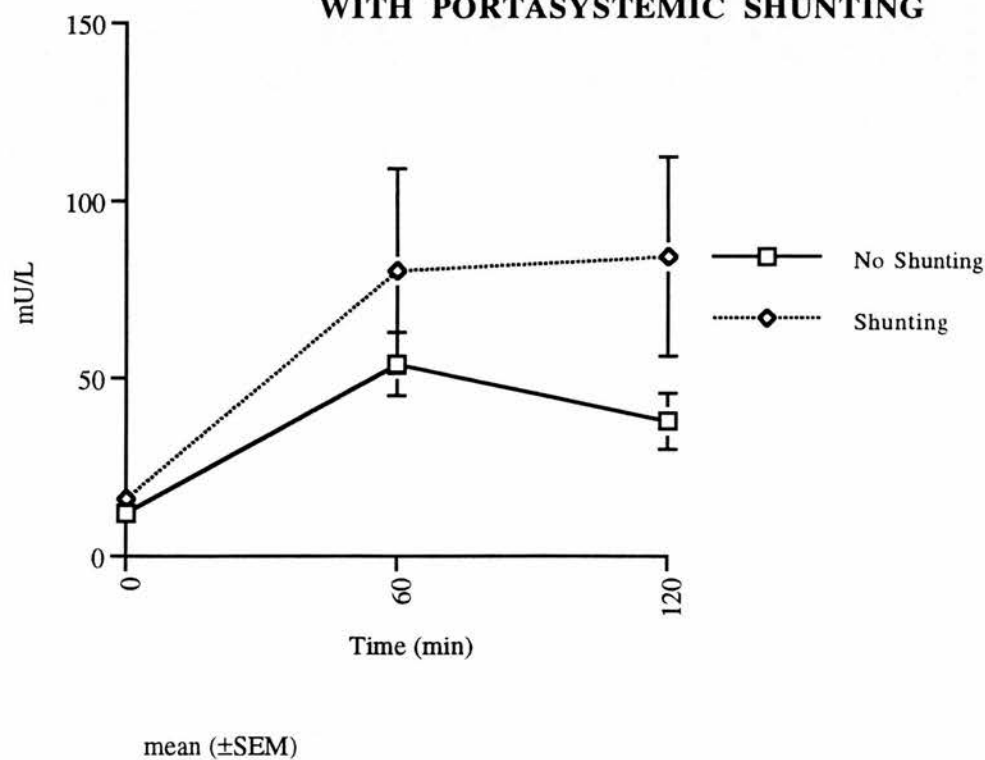
\* =  $p < 0.05$  versus controls

\*\* =  $p < 0.01$  versus controls

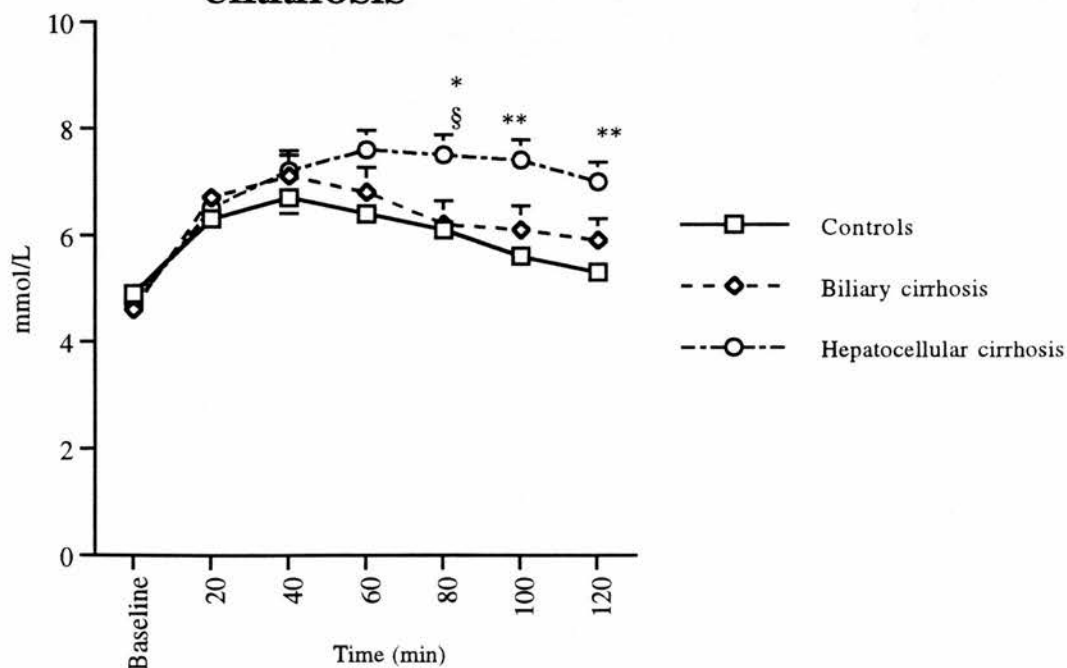
\*\*\* =  $p < 0.001$  versus controls

AUC =  $p < 0.05$  versus no shunting

**FIGURE 7.6 INSULIN RESPONSE TO FEEDING IN CIRRHOTIC PATIENTS WITHOUT AND WITH PORTASYSTEMIC SHUNTING**



**FIGURE 7.7 GLUCOSE RESPONSE TO FEEDING IN CONTROLS AND PATIENTS WITH BILIARY AND HEPATOCELLULAR CIRRHOSIS**



mean ( $\pm$ SEM)

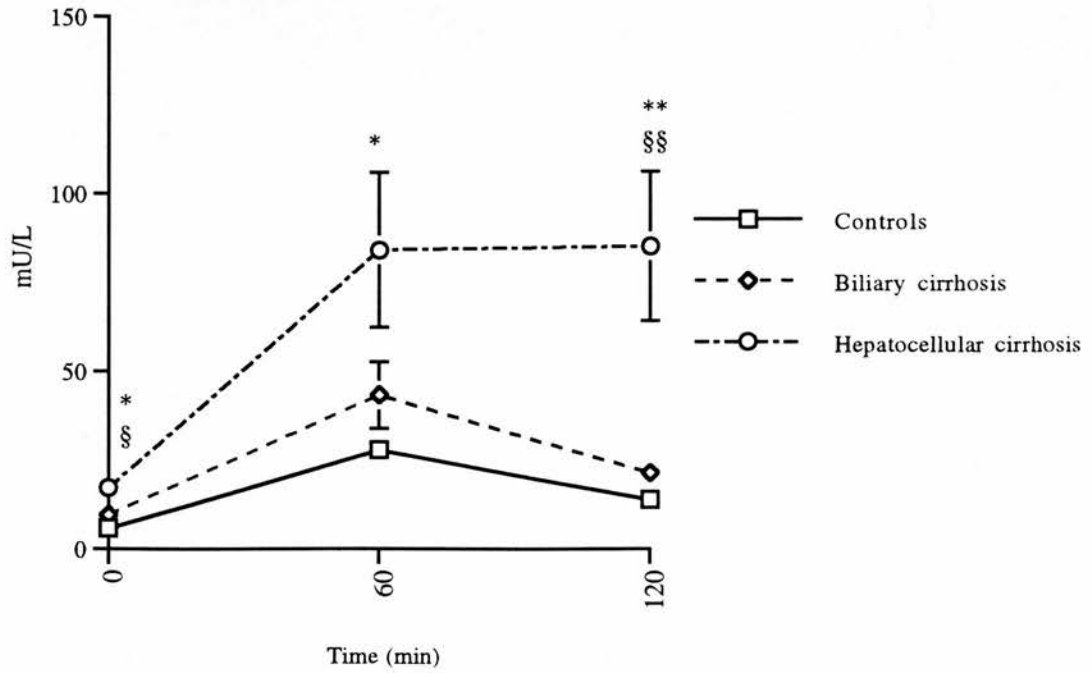
\* =  $p < 0.05$  versus controls

\*\* =  $p < 0.01$  versus controls

§ =  $p < 0.05$  versus biliary cirrhosis

AUC =  $p < 0.05$  hepatocellular versus controls

**FIGURE 7.8 INSULIN RESPONSE TO FEEDING IN CONTROLS AND PATIENTS WITH BILIARY AND HEPATOCELLULAR CIRRHOSIS**



mean ( $\pm$ SEM)

\* =  $p < 0.05$  versus controls

\*\* =  $p < 0.01$  versus controls

§ =  $p < 0.05$  versus biliary cirrhosis

§§ =  $p < 0.01$  versus biliary cirrhosis

AUC =  $p < 0.01$  hepatocellular versus controls

AUC =  $p < 0.05$  hepatocellular versus biliary cirrhosis

with HC  $17.2 \pm 2.6$  mU/l;  $p < 0.01$ ; BC  $9.6 \pm 2.2$  mU/l compared with HC,  $p < 0.05$ ). Following ingestion of the test meal, plasma insulin concentrations remained elevated in HC patients compared to controls at 60 and 120 minutes (60 min: controls  $27.9 \pm 2.9$  mU/l compared to HC  $84.2 \pm 21.8$  mU/l,  $p < 0.05$ ; 120 min: controls  $14.0 \pm 1.8$  mU/l compared to HC  $85.4 \pm 20.9$  mU/l;  $p < 0.01$ ). Two hours post-meal ingestion, a significant difference in insulin concentration between patient groups was also observed (120 min: BC  $21.5 \pm 3.5$  mU/l compared to  $85.4 \pm 20.9$  mU/l;  $p < 0.01$ ).

Area under the curve was calculated to determine the integrated glucose and insulin response to the test meal. The insulin AUC in cirrhotic patients was significantly higher than control values (insulin: controls  $2261 \pm 937$  AU compared to cirrhotics  $5575 \pm 6090$  AU,  $p < 0.05$ ). In addition the glucose AUC was significantly higher in patients with evidence of portal hypertension (glucose: no shunting  $753 \pm 112$  AU compared to  $868 \pm 198$  AU,  $p < 0.05$ ).

Differences in both the integrated glucose and insulin meal response was evident when patients were stratified by primary aetiology. In HC patients when compared with control subjects the AUC was significantly higher for both glucose and insulin (glucose: controls  $711 \pm 93.2$  AU compared to HC  $832 \pm 151$  AU,  $p < 0.05$ ; insulin: controls  $2261 \pm 937$  AU compared to HC  $7749 \pm 7429$  AU,  $p < 0.01$ ). Furthermore, the insulin AUC in HC patients was significantly greater than found in the BC group (insulin: BC  $3191 \pm 2523$  AU compared to  $7749 \pm 7429$  AU,  $p < 0.05$ ).



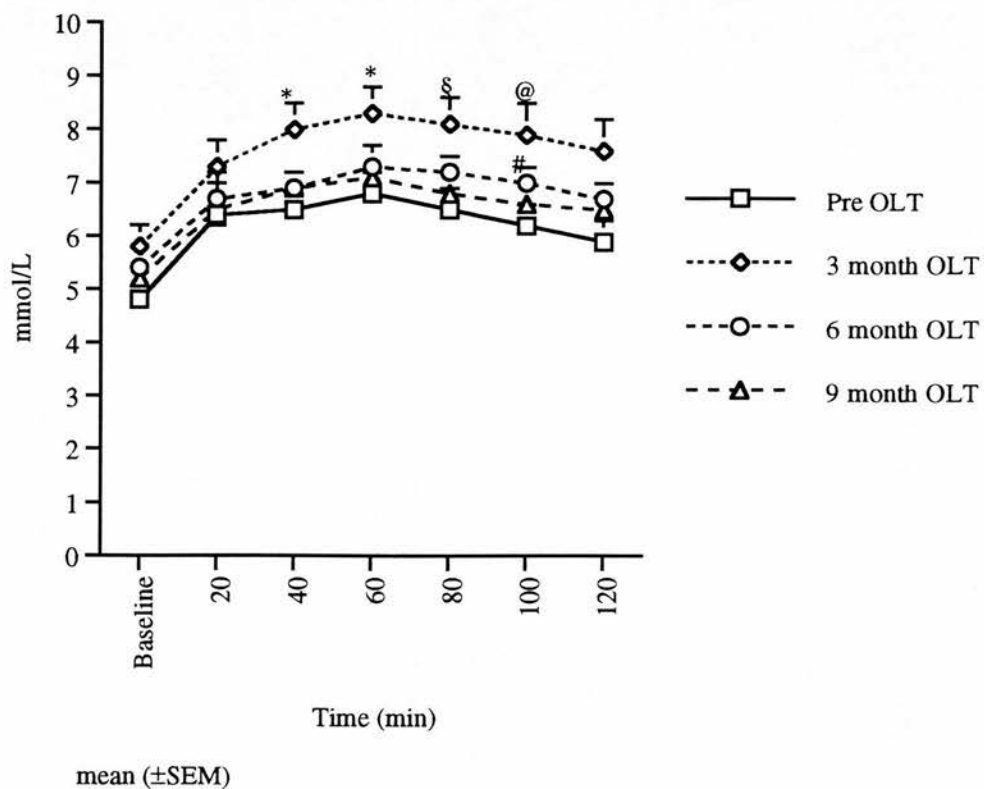
## OLT Patients

Seven male and 11 female patients (mean age  $52.8 \pm 2.4$  years) were studied following OLT. All ten BC patients were female and seven of the eight HC patients were male. There are no differences in basal blood glucose values between control subjects and patients at OLT study time points. Within the OLT group, analysis revealed that at three, six and nine months after transplantation, basal blood glucose values were significantly higher than pre-OLT values (pre-OLT:  $4.8 \pm 0.1$  mmol/l compared to 3 months OLT:  $5.8 \pm 0.4$  mmol/l;  $p < 0.05$  : pre-OLT compared to 6 months OLT:  $5.4 \pm 0.1$  mmol/l;  $p < 0.05$  : pre-OLT compared to 9 months OLT:  $5.2 \pm 0.1$  mmol/l;  $p < 0.05$ ).

From the 60 - 120 min post-prandial period, blood glucose concentration was significantly elevated in patients three months after OLT when compared to control subjects (60 min: Controls:  $6.4 \pm 0.2$  mmol/l compared to 3 month OLT:  $8.3 \pm 0.3$  mmol/l;  $p < 0.05$ : 80 min: Controls:  $6.1 \pm 0.2$  mmol/l compared to 3 month OLT:  $8.1 \pm 0.5$  mmol/l;  $p < 0.05$ : 100 min: Controls:  $5.6 \pm 0.2$  mmol/l compared to  $7.9 \pm 0.6$  mmol/l;  $p < 0.01$ : 120 min: Controls:  $5.3 \pm 0.3$  mmol/l compared to  $7.6 \pm 0.6$  mmol/l;  $p < 0.01$ ).

Significant differences in blood glucose concentration was also observed between OLT study time points (Figure 7.9). Forty and 60 minutes after meal ingestion, blood glucose concentration at three months following OLT was significantly higher than values found six and nine months after transplantation (40 min = 3 month OLT:  $8.0 \pm 0.5$  mmol/l compared to 6 month OLT:  $6.9 \pm 0.3$  mmol/l;  $p < 0.05$ : 3 month compared

**FIGURE 7.9 GLUCOSE RESPONSE TO FEEDING BEFORE AND FOLLOWING LIVER TRANSPLANTATION**

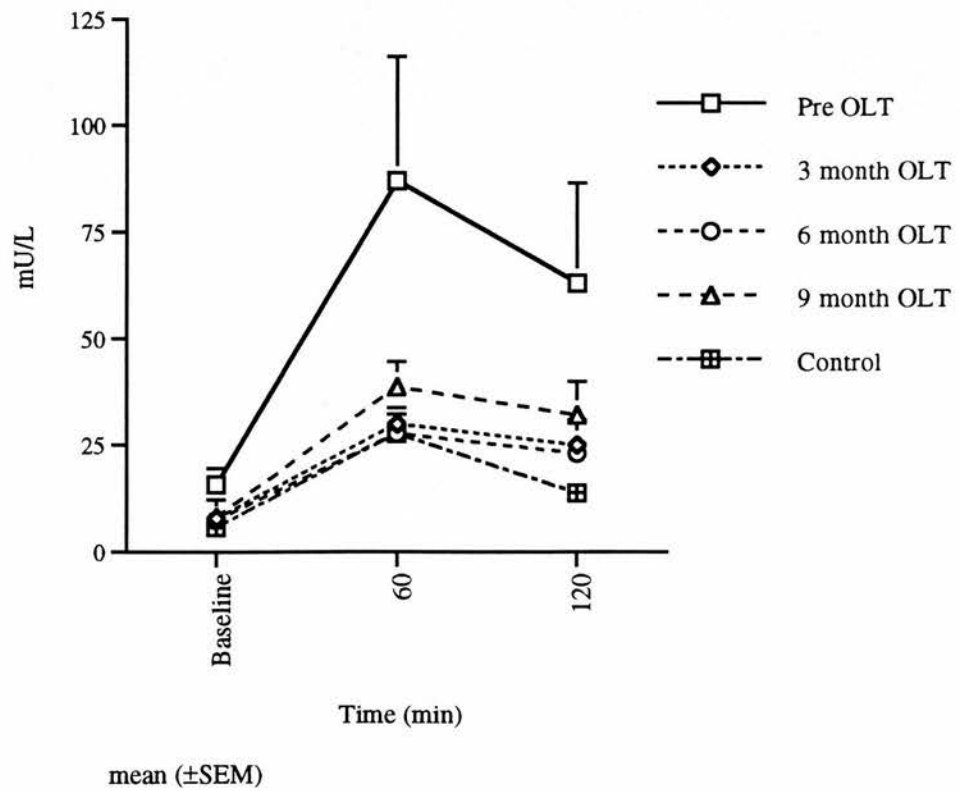


to 9 month OLT:  $6.9 \pm 0.3$  mmol/l;  $p < 0.05$ ) (60 min: 3 month OLT:  $8.3 \pm 0.5$  mmol/l compared to 6 month OLT:  $7.3 \pm 0.4$  mmol/l;  $p < 0.05$ : 3 month OLT compared to 9 month OLT:  $7.1 \pm 0.4$  mmol/l;  $p < 0.05$ ). At the 80 minute post-prandial time point, blood glucose values were significantly elevated at 3 months OLT compared to 9 months after transplant (80 min: 3 month OLT:  $8.1 \pm 0.5$  mmol/l compared to 9 month OLT:  $6.8 \pm 0.4$  mmol/l;  $p < 0.05$ ). In addition, 100 minutes after meal ingestion, blood glucose concentration was significantly higher three and six months OLT when compared to 3 month OLT (100 min: 3 months OLT:  $7.9 \pm 0.6$  mmol/l compared to 9 months OLT:  $6.6 \pm 0.3$  mmol/l;  $p < 0.05$ : 6 months OLT  $7.1 \pm 0.3$  mmol/l compared to 9 months OLT).

Post-absorptive and post-prandial plasma insulin values are shown in Figure 7.10. Basal insulin concentration was significantly elevated in pre-OLT patients when compared with controls (controls:  $5.8 \pm 0.6$  mU/l compared to pre-OLT:  $15.9 \pm 3.8$  mU/l;  $p < 0.01$ ). Sixty minutes after meal ingestion, insulin levels in pre-OLT patients remained significantly higher than control subjects (controls:  $28.9 \pm 2.9$  mU/l compared to  $87.1 \pm 29.2$  mU/l;  $p < 0.05$ ).

Differences in basal plasma insulin concentrations pre- and post-OLT were observed where elevated levels pre-transplant normalised. In response to feeding, no differences were apparent when compared pre- and post-OLT. When patients were sub-divided by primary aetiology, no difference in glucose or insulin values at three months OLT was observed. In addition, the calculated area under the curve for glucose and insulin

**FIGURE 7.10 INSULIN RESPONSE TO FEEDING BEFORE AND FOLLOWING LIVER TRANSPLANTATION**



was not significantly different from the control population or in the same patients either prior to or following transplantation.

## **Discussion**

### **Cirrhotic Patients**

In the current study, fasting cirrhotic patients were hyperinsulinaemic when compared with controls. In the late post-prandial period, blood glucose concentrations were also elevated (100 and 120 minutes). Blood glucose concentrations whilst significantly higher than controls were not indicative of DM. The incidence of DM in liver cirrhosis has been cited at 10 to 30% (Petrides and De Fronzo, 1989; Petrides et al, 1994). However, in the present study only four patients presented with DM and were excluded. The low incidence of diabetes in this study may be explained by the Scottish Liver Transplant Unit's active policy of early referral and it may be that these stable cirrhotic patients who are amenable to assessment represent a different cohort to those those admitted for management of a disease related complication. Indeed, no patient in the present study was admitted for management of an acute complication of their liver disease and this finding may account for the more moderate disturbances of carbohydrate metabolism than previously reported.

The impact of portasystemic shunting on glucose and hyperinsulinaemia remains one of debate. Kruszynska et al (1993) suggests the early elevation in blood glucose, seen in response to an oral

glucose tolerance test is a consequence not only of reduced hepatic mass but of a reduced parasympathetic endocrine response. In the present study patients were sub-divided by the presence or not of portasystemic shunting and those with portal hypertension were shown to be hyperglycaemic from 60 - 120 minutes when compared to those patients without portal hypertension. However nine out of the 14 (65%) patients with shunting had HC and this may bias results as a significant disturbance in the glucose response to feeding was seen in HC patients. No significant differences were observed in the insulin response to feeding when portasystemic shunting was considered.

The effect of portasystemic shunting on carbohydrate metabolism is equivocal and Bosch et al (1984) used hepatic vein catheterisation to assess the contribution of portasystemic shunting and impaired hepatic extraction to the hyperinsulinaemia of cirrhosis. These investigators reported that hyperinsulinaemia was the result of the shunting of pancreatic venous blood to the systemic circulation. Whilst portasystemic shunting may contribute to the hyperinsulinaemia and hyperglycaemia of cirrhosis, its impact may have been overstated given that postprandial hyperinsulinaemia is not observed in patients with portal vein thrombosis and normal liver function (Johnson et al, 1978). This view is supported by Taylor et al (1985) who in an euglycaemic/hyperinsulinaemic clamping study suggested that receptor/post-receptor defects are more important than portal hypertension in the pathogenesis of insulin resistance.

Many studies have considered the cause of glucose intolerance in cirrhotic populations but generally these have been performed in small

heterogeneous groups. Stratification of cirrhotic patients is important as disease severity and aetiology could influence glucose and insulin metabolism. In the current study when patients were stratified for disease severity using the modified Child's Pugh score, no significant differences in glucose or insulin concentrations were observed and could be related to the stable clinical state of this population. Interestingly, Muller et al (1992b), in a euglycaemic/hyper-insulinaemic clamping study which examined the mechanisms of insulin resistance, observed no differences in glucose and insulin responses between patients classified as Child's Pugh A and Child's Pugh B. In this study no patients were classified as Child's Pugh C.

The primary aetiology of cirrhosis was also examined in the present study. This stratification highlighted differences in the insulin and glucose in the fasted and fed state. Hepatocellular cirrhotic patients exhibited an elevation in blood glucose concentration in response to the test meal that were significantly different from controls and BC patients. However, when the integrated glucose meal response was determined only differences between HC patients and control group were apparent. It should be emphasised that whilst HC patients blood glucose concentrations were elevated, the response curve was not indicative of overt DM. The glucose dose given in the test meal was comparable to a oral glucose tolerance test and supplied 3.6 g of carbohydrate per kg body weight. Plasma insulin concentration and the integrated meal response was also shown to be significantly higher in HC patients compared to controls as well as between HC and BC patients. This suggests that

disturbances of glucose metabolism are of a greater magnitude in patients whose primary aetiology is associated with intrinsic hepatocellular damage but this cannot be confirmed. Few metabolic studies have examined groups large enough to stratify their patients by disease, although Taylor et al (1985), in a study that examined insulin action in cirrhosis found that patients with cholestatic disease were less insulin-resistant than patients with alcoholic or cryptogenic cirrhosis. In the present study the increased basal insulin and glucose/insulin response to feeding in the HC patients may reflect an adaptive response to defective glycogen storage. Results suggest that in HC patients the glucose in the meal must be immediately oxidised resulting in a concomitant but short term decrease in lipid oxidation (Eriksson et al, 1989).

Whilst the understanding of altered glucose metabolism in response to feeding has previously been explored (Nolte et al, 1995) what has not been considered is the effect of hyperinsulinaemia on ingestive behaviour. Normally, the presence of food in the gastrointestinal tract stimulates the release of gastrointestinal hormones (i.e. gastric-inhibitory-peptide, CCK, gastrin, secretin). The cephalic and post ingestive humoral response to feeding includes the release of insulin. These humoral messengers reinforce neural signals which converge in the brain stem and subsequently act to influence ingestive behaviour. Insulin has been shown to act through the central nervous system as a satiety mediator and acts by reducing meal size (Van der Weele et al, 1980; Posner, 1987). Whether the satiety effect of insulin depends upon glucose is not known but insulin infusions in rats fed low carbohydrate, high fat diet did not



depress food intake and suggests glucose may be involved (Arase et al, 1988).

It may be postulated that the disturbed glucose/insulin response to feeding seen in cirrhotic patients and in particular those with disease of hepatocellular origin could contribute to reductions in food intake.

### **OLT Patients**

Hyperglycaemia and new onset DM (insulin dependent) is a well recognised complication of solid organ transplantation with the prevalence reported as ranging from 4 - 20% (Jindal, 1994). Usually transient in nature, post-transplantation DM (Tabasco-Mingullan et al, 1993) has been related to immunosuppressive drug therapy and more precisely to the dose and duration of steroid therapy (Navasa et al, 1996). It is now widely accepted that the incidence of post-transplantation DM can be decreased by the early withdrawal of steroid therapy and this has been shown to be a safe treatment strategy in OLT patients (Gomez et al, 1998). Interestingly, the success rate of early steroid withdrawal in transplantation appears to be more successful in OLT compared to other solid organ transplants (heart, lung, kidney) and may be related to the fact that the liver is an immunologically privileged organ (McDiarmid et al, 1995). In the 18 patients studied longitudinally, basal OLT blood glucose values were higher after transplantation but it should be emphasised that following OLT these values (3, 6 and 9 months OLT) were not significantly different from the control group. Roth et al (1989) diagnosed post-transplant diabetes using fasting blood glucose concentrations and

reported a prevalence twice that of the present study. In the current study there was a trend for fasting blood glucose concentrations to decrease from the third to the ninth month after transplantation.

During the second half of the post-prandial period (60 - 120 minutes), the blood glucose response was significantly elevated in patients three months after OLT when compared with control subjects. In addition, this three month OLT post-prandial elevation in blood glucose was also significantly greater than that observed in the same patients nine months after transplantation. It is likely that the increased glucose response to feeding seen in the early post-operative period is attributable to introduction of steroid therapy (Pirsch et al, 1983). It has been shown that early steroid withdrawal results in improved glucose tolerance and for those patients with post-transplant DM a significant reduction in insulin requirements (Hricik et al, 1991).

The synergistic effect of immunosuppression on glucose tolerance cannot be overlooked. For example, in a study of renal transplant recipients, it was suggested that CsA (cyclosporin A) may reduce the clearance of steroids (Ost, 1984). Cyclosporin A, tacrolimus (FK506) and steroids have been implicated in the development of post-transplantation DM and appears to be the result of decreased insulin secretion, insulin resistance and damage to the pancreatic beta - cells (Jindal, 1994). However, in the present study, in uncomplicated OLT transplant recipients from six months OLT, fasting and fed glucose values were not significantly different to control subjects.

No differences in pre- and post-OLT plasma insulin concentrations were observed and may be accounted for by the fact that of those patients submitted to OLT, ten had cholestatic disease and eight HC disease. In the present study, an elevated basal and post-prandial response to feeding was observed in HC patients. Following transplantation, plasma insulin concentrations were not significantly different from controls or in the same patients at different OLT time points.

In the preceding section focusing on cirrhotic patients, it was suggested that the centrally mediated satiety effects of insulin may be exaggerated and could affect dietary intake but this was not apparent after liver transplantation.

## CHAPTER 8

### ENERGY AND MACRONUTRIENT INTAKE IN LIVER CIRRHOSIS AND LIVER TRANSPLANT RECIPIENTS

Few studies have quantitatively examined nutritional intake in patients with liver cirrhosis and these have almost exclusively focused on patients with hepatocellular cirrhosis (Morgan et al, 1982; Egan et al, 1985; Cabre et al, 1990; Campillo et al, 1997). However, with the exception of the study by Campillo and co-workers (1997), suboptimal spontaneous nutritional intakes have been observed in the majority of studies that have considered nutritional intake in liver cirrhosis (Cabre et al, 1990). Neilsen et al (1993) reported that in undernourished cirrhotic patients, dietary intakes were only 61% of a comparable control population. They concluded that ensuring cirrhotic patients consume an adequate nutritional intake is a major problem when attempting to improve nutritional status.

The increased understanding of the metabolic demands of liver disease has resulted in the publication of European Dietary Guidelines (Plauth et al, 1997) on nutritional requirements in liver disease. As a consequence nutritionists can now quantify dietary targets for patients with liver disease and if consumed, these regimens should prove clinically effective.

The mechanisms involved in the aetiology of anorexia in liver disease are unknown but may in part be due to the fact that the complex interactions between altered metabolism and nutritional intake are not

well defined. The physiological sequelae of progressive liver damage and consequent aberrant metabolism may result in alterations in the role of the liver as a metabolic sensor and may effect changes in ingestive behaviour and influence nutritional intake. In order to examine factors associated with anorexia in chronic liver disease, spontaneous dietary intake was estimated in a group of stable cirrhotic patients.

Following transplantation, obesity has been reported in OLT patients and the magnitude of weight gain has been shown to exceed that of patients pre-illness weight (Davidson et al, 1998). The presence of obesity in OLT may exacerbate other common post-transplantation complications such as hyperlipidaemia, hypertension and cardiovascular disease (Munoz et al, 1991) and given the central role of hepatic afferent signalling in ingestive behaviour, it is surprising that the nutritional intake of denervated OLT patients has not been examined. This study also examined the dietary intake of a sub-group of patients with cirrhosis who were subsequently transplanted at three, six and nine months following discharge from hospital.

## **Patients and Methods**

### **Patients**

Sixty seven cirrhotic patients (35 BC patients and 32 HC patients) were recruited.

Twenty three cirrhotic patients were submitted to OLT and had an uncomplicated post operative recovery these patients were reviewed on a

three monthly basis on three occasions after discharge from hospital. Eighteen healthy volunteers were also recruited.

## Methods

Food diaries were used to estimate energy and macronutrient intake (recording two week days and one day at the weekend) and were kept by subjects in the week following discharge from hospital. The methods used fully described in Chapter 2. No patient with chronic liver disease was on a therapeutic diet.

## Results

### Cirrhotic Patients

Characteristics of this cohort are shown in Table 3.1. Three day dietary diaries were analysed on 52 of the 67 cirrhotic patients and 16 of the 18 controls, a return rate of 78% and 89% for patients and controls respectively. The validity of the dietary diary technique was determined in the control group by comparing protein intake (expressed as nitrogen) with nitrogen output. A significant relationship between these variables was observed ( $r = 0.562$ ;  $p < 0.05$ ).

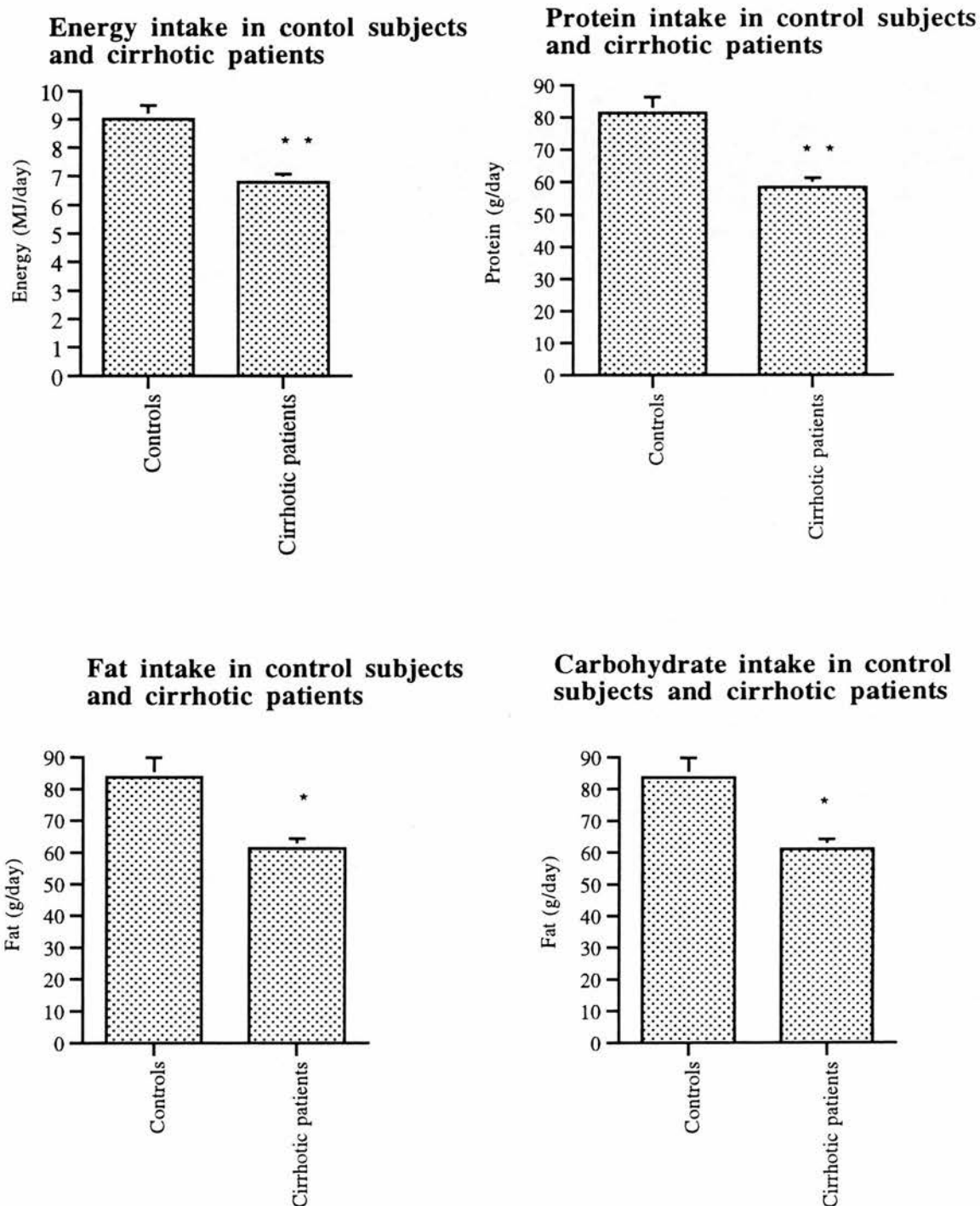
The cirrhotic patients had significantly lower intakes of energy when compared with control subjects (controls:  $9.0$  (2145kcal)  $\pm 0.45$  MJ/day versus cirrhotic patients:  $5.8$  (1617 kcal)  $\pm 0.29$  MJ/day;  $p < 0.01$ ), protein (controls:  $81.3 \pm 5.0$  g/day versus cirrhotic patients:  $58.3 \pm 2.9$  g/day;  $p < 0.001$ ), fat (controls:  $83.7 \pm 6.2$  g/day versus cirrhotic patients  $61.1 \pm 3.2$  g/day;  $p < 0.01$ ) and carbohydrate (controls:  $259.4 \pm 11.4$  g/day

versus cirrhotic patients  $202.3 \pm 10.1$  g/day;  $p < 0.01$ ) (Figure 8.1). Resting energy balance (energy input minus resting energy expenditure) was significantly lower in cirrhotic patients when compared with the control subjects (controls:  $2.4$  (584 kcal)  $\pm 0.36$  MJ/day versus cirrhotic patients:  $1.0$  (245 kcal)  $\pm 0.30$  MJ/day;  $p < 0.05$ ).

When patients were stratified for severity of liver disease by Child's Pugh score, no significant differences were shown in energy and macronutrient intake between Child's Pugh A ( $n = 12$ ), B ( $n = 23$ ) and C ( $n = 17$ ) patients (Figure 8.2). In addition, there were no differences in energy balance between these groups (Child's Pugh A =  $-0.228 \pm 1.24$ ; Child's Pugh B =  $-1.03 \pm 1.18$ ; Child's Pugh C =  $1.44 \pm 1.11$ ; ns).

The energy and macronutrient intake of control subjects and patients classified by primary aetiology was examined. When compared with controls, the energy intake in both BC ( $n = 26$ ) and HC ( $n = 26$ ) cirrhotic patients was significantly lower (controls versus BC:  $7.0$  (1678 kcal)  $\pm 0.40$  MJ/day;  $p < 0.05$ : controls versus HC:  $6.5$  (1565 kcal)  $\pm 0.39$  MJ/day;  $p < 0.01$ ). Protein intake was also significantly lower in patient groups when compared with controls (controls versus BC:  $61.4 \pm 3.9$  g/day;  $p < 0.05$ : controls versus HC:  $55.6 \pm 4.1$  g/day;  $p < 0.001$ ). However, the dietary intake of fat and carbohydrate when compared with the control group was significantly lower only in the HC group (fat: controls versus HC:  $61.3 \pm 4.4$  g/day;  $p < 0.05$ ) (carbohydrate: controls versus HC:  $195.1 \pm 13.3$  g/day;  $p < 0.05$ ) (Figure 8.3). Additionally, resting energy balance was significantly lower in the HC patients when compared with

**FIGURE 8.1 ENERGY AND MACRONUTRIENT INTAKE IN CONTROL SUBJECTS AND CIRRHOTIC PATIENTS**



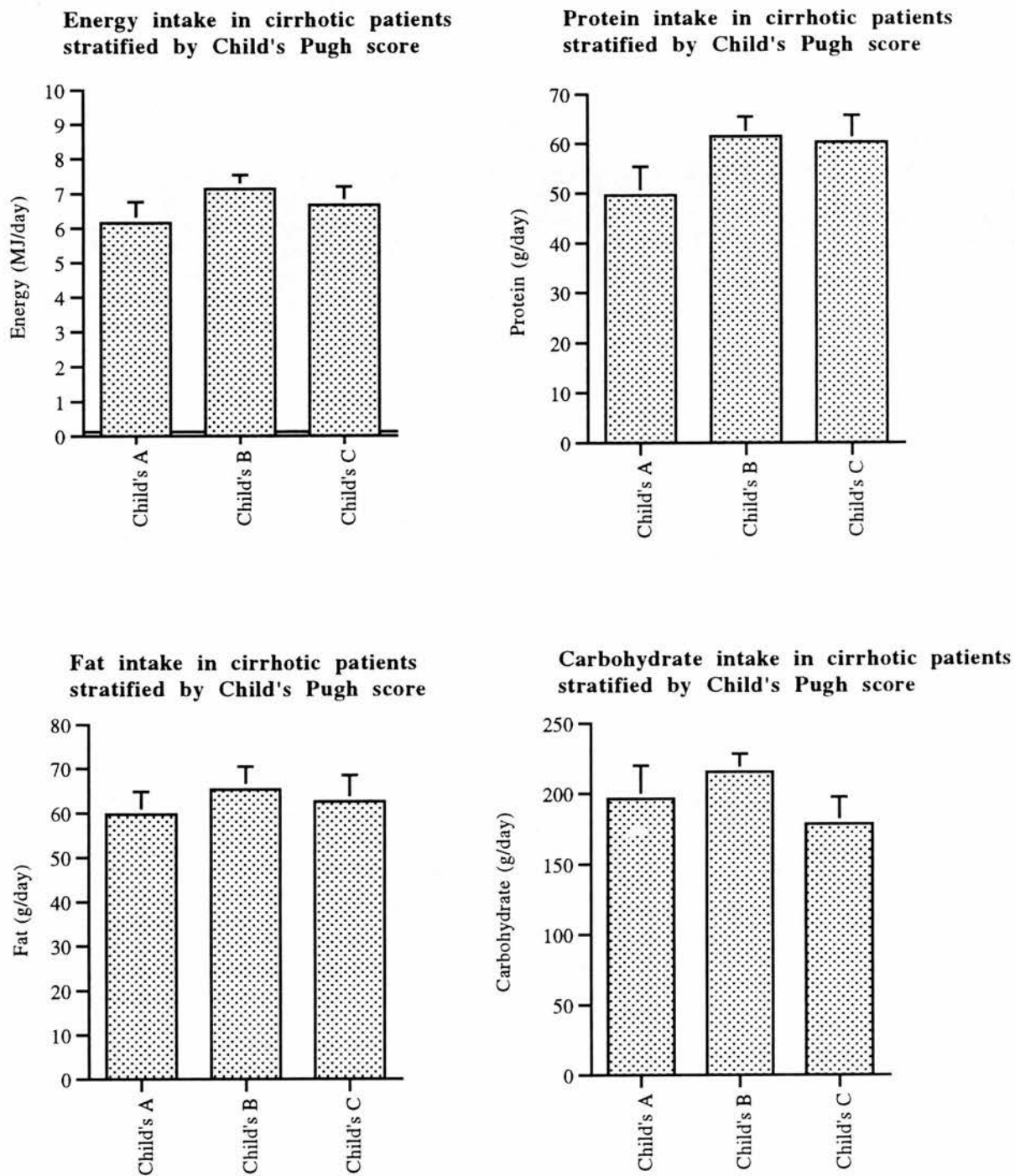
mean ( $\pm$  SEM)

\* =  $p < 0.01$  versus control subjects

\*\* =  $p < 0.001$  versus control subjects

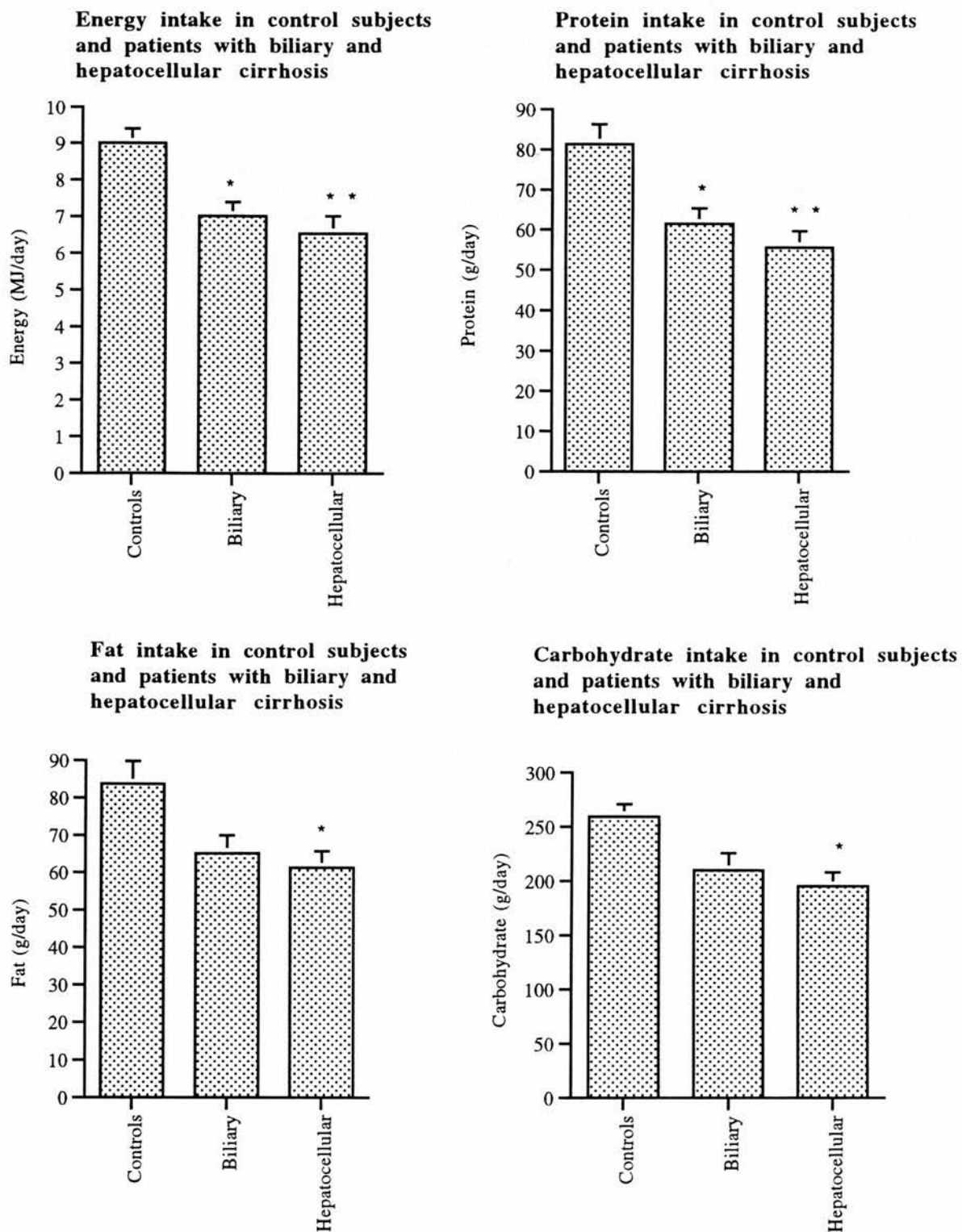


**FIGURE 8.2 ENERGY AND MACRONUTRIENT INTAKE IN CIRRHOTIC PATIENTS STRATIFIED BY DISEASE SEVERITY**



Mean ( $\pm$ SEM)

**FIGURE 8.3 ENERGY AND MACRONUTRIENT INTAKE IN CONTROL SUBJECTS AND PATIENTS STRATIFIED BY AETIOLOGY**



Mean ( $\pm$ SEM)

\* =  $p < 0.05$  versus control subjects

\*\* =  $p < 0.01$  versus control subjects

controls (controls versus HC:  $0.51 (124 \text{ kcal}) \pm 0.42 \text{ MJ/day}$ ;  $p < 0.05$ ) (Figure 8.4).

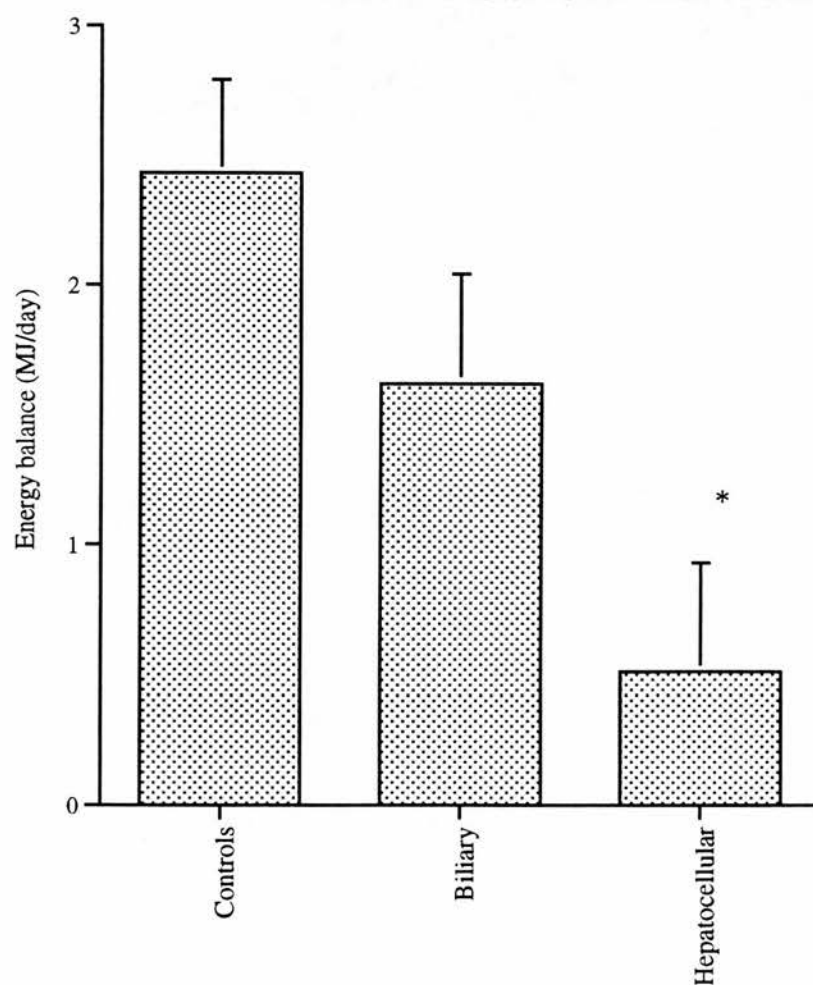
When energy and macronutrient intakes were expressed as a unit of body weight (per kg body weight), although depressed when compared with controls, they were not significantly different. Additionally there were no differences in nitrogen balance (including 3 gm insensible losses) when disease severity or primary aetiology was examined (Figure 8.5). There was no difference in the number of eating episodes between control subjects (controls =  $4.9 \pm 0.23 / \text{day}$ ), BC patients (BC =  $4.5 \pm 0.22 / \text{day}$ ) and HC patients (HC =  $4.4 \pm 0.21$ ).

### **OLT patients**

Characteristics of OLT patients are shown in Table 3.7. For each study time point, the return rate for dietary diaries was 91% ( $n = 21$  out of 23). Compared with pre-OLT values, significant increases in energy and macronutrient intakes were observed (Table 8.1). Additionally six months after OLT, these patients consumed significantly more energy than control subjects (Controls:  $9.0 \pm 0.45 \text{ MJ/day}$  versus  $10.2 \pm 0.62 \text{ MJ/day}$ ,  $p < 0.05$ ). The intake of dietary fat was greater from three months post-OLT (Fat: Controls  $83.7 \pm 6.2 \text{ g/day}$  versus three months OLT:  $106.2 \pm 10.1 \text{ g/day}$ ,  $p < 0.05$ ; Controls versus six months OLT:  $110.4 \pm 7.5 \text{ g/day}$ ,  $p < 0.01$ ; Controls versus nine months OLT:  $101.6 \pm 7.1 \text{ g/day}$ ,  $p < 0.05$ ).

Differences in energy and macronutrient intake were also apparent when dietary intake data were normalised for body weight (Figure 8.6). Compared with pre-OLT values, there were significant increases in energy

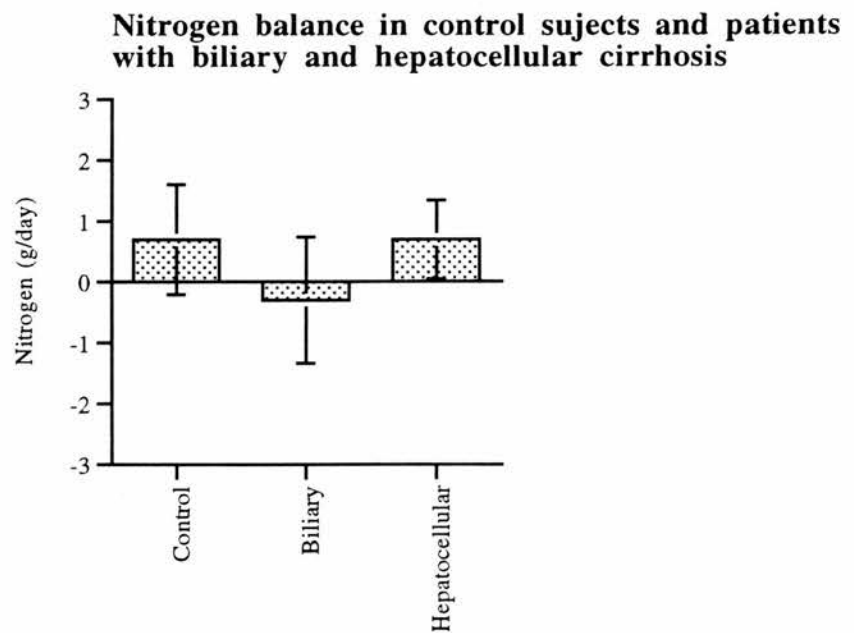
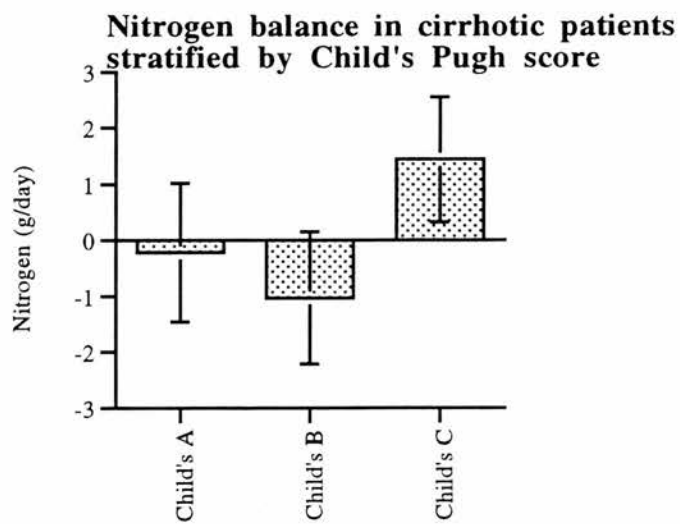
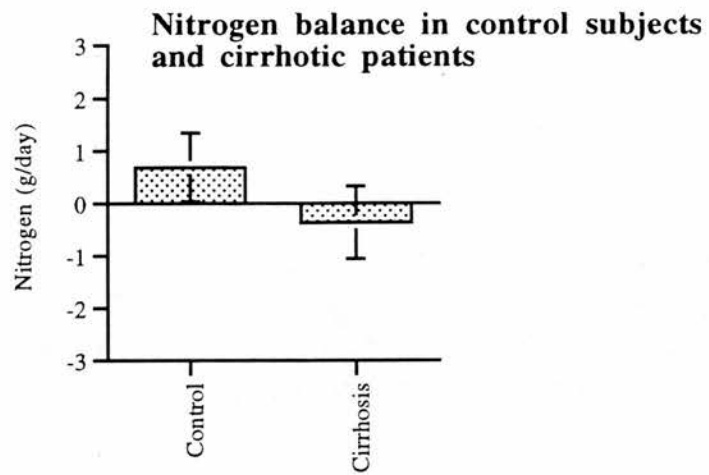
**FIGURE 8.4 ENERGY BALANCE IN CONTROL SUBJECTS AND PATIENTS STRATIFIED BY AETIOLOGY**



Mean ( $\pm$ SEM)

\* =  $p < 0.05$  versus control subjects

**FIGURE 8.5 NITROGEN BALANCE**



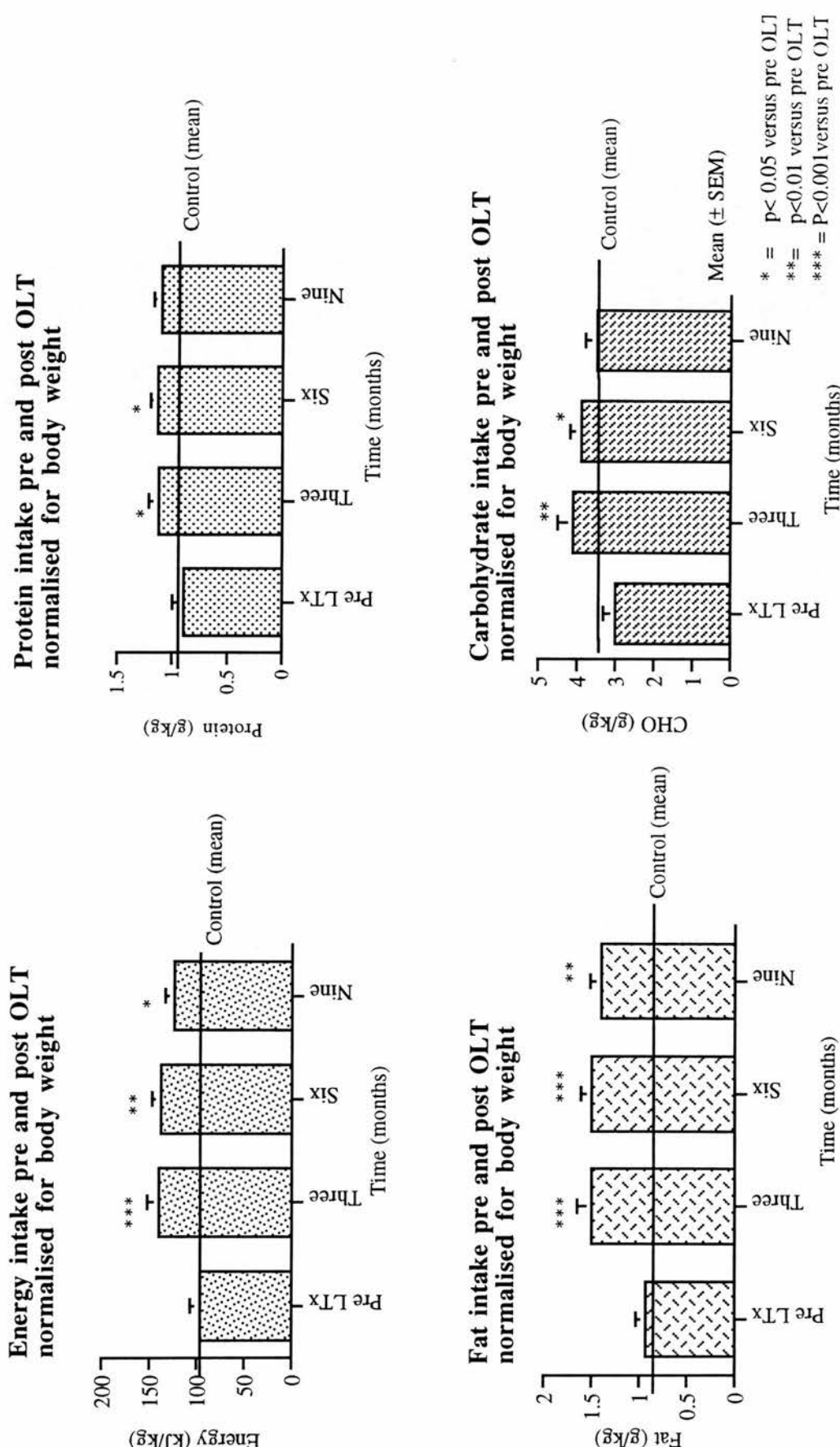
Mean ( $\pm$ SEM)

**TABLE 8.1**  
**ENERGY AND MACRONUTRIENT INTAKE IN CIRRHOTIC PATIENTS BEFORE AND FOLLOWING LIVER TRANSPLANTATION**

	Energy (MJ/day) (kcal/day)	Protein (g/day)	Fat (g/day)	Carbohydrate (g/day)
Pre-OLT patients	6.40 (0.52) (1542)	60.3 (5.6)	62.3 (5.4)	199.0 (17.2)
3 months OLT	9.9 (0.81) <sup>a</sup> (2360)	79.3 (5.5) <sup>b</sup>	106.2 (10.1) <sup>a</sup>	288.0 (27.1) <sup>b</sup>
6 months OLT	10.2 (0.62) <sup>a</sup> (2444)	84.9 (4.3) <sup>b</sup>	110.4 (7.5) <sup>a</sup>	292.4 (8.9) <sup>b</sup>
9 months OLT	9.4 (0.59) <sup>b</sup> (2227)	83.4 (4.0) <sup>b</sup>	101.6 (7.1) <sup>a</sup>	261.8 (20.0) <sup>c</sup>
Controls	9.0 (0.45) <sup>d</sup> (2142)	81.3 (5.0)	83.7 (6.2) <sup>e,f,g</sup>	259.4 (11.4)

Mean ( $\pm$ SEM)	a = p<0.001 versus pre-OLT	b = p<0.01 versus pre-OLT	c = p<0.05 versus pre-OLT	d = p<0.05 versus 6 months OLT	e = p<0.05 versus 3 months OLT	f = p<0.01 versus 6 months OLT	g = p<0.05 versus 9 months OLT
Comparisons with controls - Mann Whitney U test							

**FIGURE 8.6 ENERGY AND MACRONUTRIENT INTAKE BEFORE AND FOLLOWING LIVER TRANSPLANTATION**

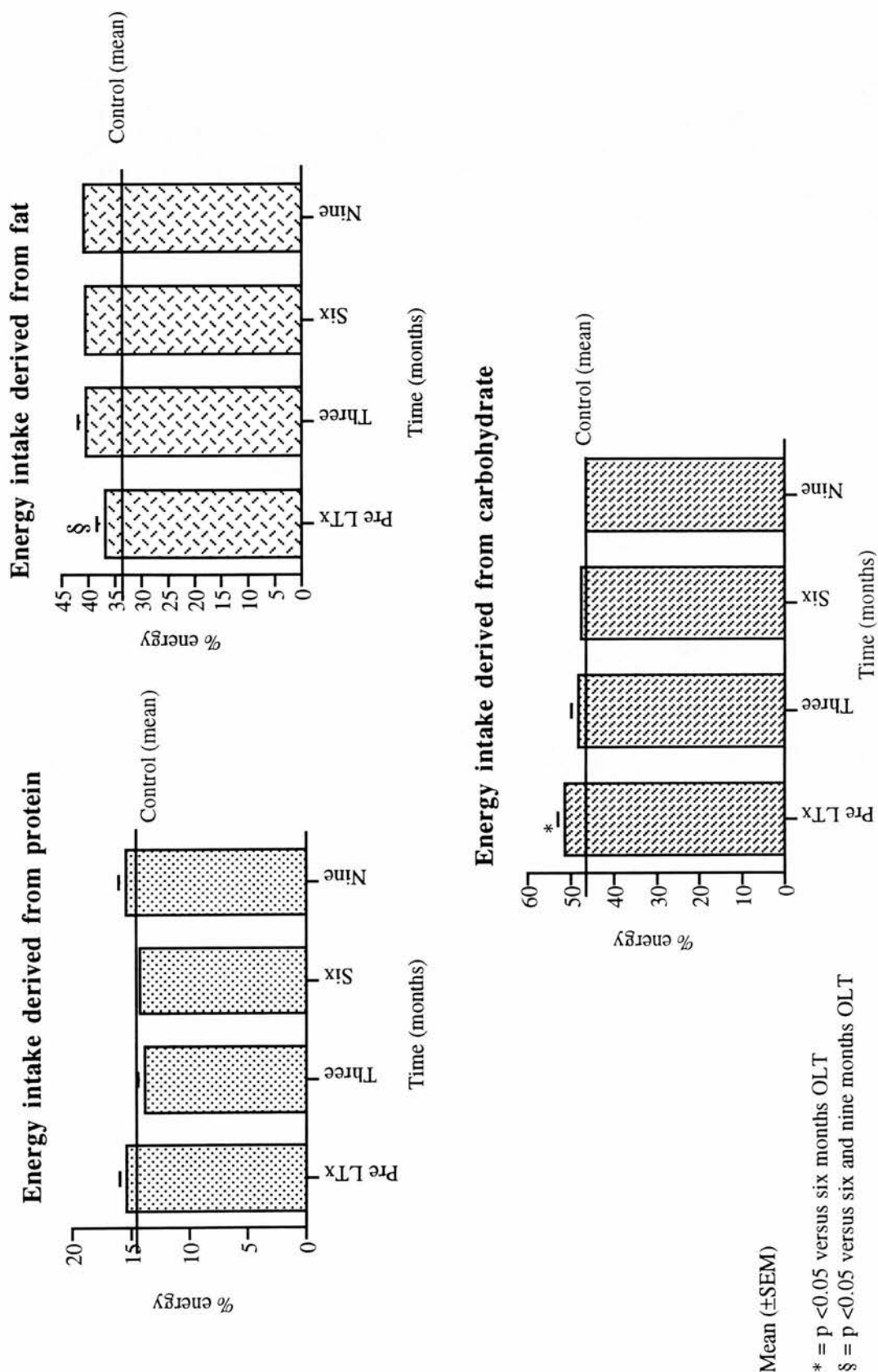


(pre-OLT:  $96.6 \pm 10.4$  kJ/kg versus three months OLT:  $139.9 \pm 12.1$  kJ/kg,  $p < 0.01$ : pre-OLT versus six months OLT:  $137.7 \pm 9.7$  kJ/kg,  $p < 0.01$ : pre-OLT versus nine months OLT:  $123.5 \pm 9.7$  kJ/kg,  $p < 0.05$ ), protein (pre-OLT:  $0.89 \pm 0.10$  g/kg versus three months OLT:  $1.12 \pm 0.09$  g/kg,  $p < 0.05$ : pre-OLT versus six months OLT:  $1.14 \pm 0.07$  g/kg,  $p < 0.05$ ), fat (pre-OLT:  $0.93 \pm 0.11$  g/kg versus three months OLT:  $1.50 \pm 0.15$  g/kg,  $p < 0.001$ : pre-OLT versus six months OLT:  $1.48 \pm 0.11$  g/kg,  $p < 0.001$ : pre-OLT versus nine months OLT:  $1.34 \pm 0.12$  g/kg,  $p < 0.01$ ) and carbohydrate (pre-OLT:  $2.97 \pm 0.32$  g/kg versus three months OLT:  $4.06 \pm 0.39$  g/kg,  $p < 0.01$ : pre-OLT versus six months OLT:  $3.92 \pm 0.29$  g/kg,  $p < 0.05$ ). Six and nine months after OLT dietary fat intake expressed as a unit of body weight exceeded that of control subjects (Controls:  $1.0 \pm 0.10$  g/kg versus six months OLT:  $1.48 \pm 0.11$  g/kg,  $p < 0.01$ : Controls versus nine months OLT:  $1.34 \pm 0.12$  g/kg,  $p < 0.05$ ).

Prior to and following OLT the profile of energy derived from macronutrients was also examined and nine months after OLT a significant increase in the contribution from fat to total energy intake was observed and was accompanied by a significant decrease in the energy derived from carbohydrate (Figure 8.7). When OLT patients were compared with controls, the percentage energy derived from fat was significantly higher in patients at three (controls:  $34.9 \pm 5.2\%$  versus three months OLT:  $40.4 \pm 6.8\%$ ,  $p < 0.01$ ), six (controls: versus six months OLT:  $40.5 \pm 4.2\%$ ,  $p < 0.01$ ) and nine months after transplantation (controls: versus nine months OLT:  $40.9 \pm 5.5\%$ ,  $p < 0.01$ ).



**FIGURE 8.7 CONTRIBUTION OF MACRONUTRIENTS TO TOTAL ENERGY INTAKE BEFORE AND FOLLOWING LIVER TRANSPLANTATION**



At the six month study time point, 17 out of 23 (74%) patients were prescribed an energy restricted dietary regimen.

## **Discussion**

### **Cirrhotic Patients**

The present study examined spontaneous food intake in a group of stable cirrhotic patients. In order to minimise the environmental and behavioural influences of hospitalisation, subjects recorded their dietary intakes at home and a day at the weekend was also included in the diary period to allow for any day of the week effect (Beaton et al, 1979). The use of the three day dietary diary in the current study was valid as is evidenced by the relationship between nitrogen output and nitrogen input ( $p < 0.05$ ) and has previously been shown to be of value in the estimation of energy and macronutrient intake (Gibson, 1990).

As with other studies, the current investigation demonstrated that cirrhotic patients had a significantly lower nutritional intake than control subjects. The average daily energy intake was only 75% of that achieved by the control group but was greater than that found by Neilsen et al (1993) and Egan et al (1985) where energy intake of their hospitalised patients was 61% and 50% of their control groups respectively. The reason for the higher energy intake in the present study may be that patients were documenting their intakes at home and were therefore consuming familiar and acceptable foods. Additionally, no patient in the current study was admitted for an acute exacerbation of their disease nor an infective complication which could have affected appetite.

When patients were stratified using Child's Pugh classification, to examine the effect of disease severity on nutritional intake, no differences in energy or macronutrient intake were evident. It should be remembered that the Child's Pugh score is a dynamic clinical index of disease severity but not nutritional status. Indeed the studies of Campillo et al (1997) and Neilsen et al (1993) have also shown that disease severity had no effect on nutritional intake.

However, when patients were examined by primary aetiology, differences in energy and macronutrient intakes were observed. When compared with controls, energy intakes were significantly lower in the BC group but the level of energy intake was lowest in the HC cirrhotic group. It is unlikely that female bias in the BC cirrhotic group has influenced the differences in energy intakes observed between patients group. Indeed it might be expected that the BC rather than the HC patients would have the lowest energy intake, as females have a lower lean body mass than males and as a result a lower energy requirement. Furthermore, it could be inferred that when compared with controls the depressed energy intakes in BC cirrhotic and HC group is merely a reflection of the reduced body mass that may accompany chronic liver cirrhosis. However, in the present study, body mass index was not different between patient groups and controls and, although fat mass was significantly lower in the HC group, lean body mass was preserved (Chapter 3). When energy and macronutrient intake was expressed as a unit of body mass (kg body weight), although intakes of HC patients were lower than controls, they failed to reach significance. However, their depressed intake per unit

body mass, if considered cumulatively, may result in a negative energy balance and consequent depletion of endogenous energy reserves as seen in the present study.

The level of patient activity was not examined in the current study as completion of exercise diaries was thought to be too onerous for the patient group. Further studies that consider activity levels in these patients would improve our understanding of energy dynamics in liver disease (energy balance = energy in - energy out (resting + thermogenesis + activity)).

During assessment for OLT, the SLTU dietitian gives patients dietary advice and uses ESPEN Guidelines (European Society of Parenteral and Enteral Nutrition) (Plauth et al, 1997) as the template for prescribing individual dietary regimens for patients with liver disease. These guidelines suggest that 40 kcal/kg body weight would provide sufficient energy to improve nutritional status and a protein intake of 1.4 g/kg body weight would achieve nitrogen balance in patients with chronic liver disease. Despite professional dietary advice cirrhotic patients in this study and in particular those with HC disease were unable to achieve target prescriptions for either energy or protein. Although dietary protein is an important constituent of structural, functional and transport proteins, it is not considered a major contributor of total energy intake and will not be examined in depth in this thesis.

The mismatch between patient's actual and prescribed intakes serves to highlight the fact that whilst a nutritionally adequate diet (including energy dense supplements) may be prescribed for these

patients, their ad libitum intake is suboptimal as is evidenced by the HC group and may contribute to undernutrition. Interestingly, Kondrup and Muller (1997) in a review of eight studies on patients with chronic liver disease concluded that when nutritional support (parenteral, enteral and oral regimens) was implemented a decrease in mortality was associated with increased energy and protein intakes. However, in this review oral nutritional intakes were suboptimal. These findings are in agreement with the present study but at odds with the work of Campillo et al (1997) who examined oral intake in a group of 55 alcoholic cirrhotics. In Campillo and colleagues (1997) study all patients were hospitalised for a disease related complication and a third had evidence of an infective complication. Patients were included in the study up to five weeks after admission and then followed for a 30 day study period. The investigators observed small improvements in nutritional status (Child's Pugh A, B and C) defined by anthropometric indices over the study period despite half of the patients being prescribed a low sodium diet, which in itself is unpalatable. The reason for the improved nutrient intake and nutritional status in this study could merely be a reflection of an improved clinical state. The average weight of these patients was 59.5 kg and energy consumption averaged 10.0 MJ/day (2391 kcal/day) which equates to around 40kcal/kg and is a substantive intake for these hospitalised patients. Indeed, the majority of studies in this area support our own findings in that undernutrition in cirrhosis is common and patients find it difficult or impossible to maintain an adequate nutritional intake (Achord, 1987; Cabre et al, 1990).

A unique aspect of the present study is that it has not solely examined patients with alcoholic or HC disease but also considered patients with cholestatic cirrhosis. In the current study the energy and macronutrient intake of the HC group was lowest. Whilst the metabolic sequelae of chronic liver disease has been widely investigated, few studies have quantitatively and qualitatively examined the nutrient intake of patients with cirrhosis and their ability to meet prescribed intakes. One study that has gone some way to examining a nutritional strategy that may improve intake was by Verboeket et al (1995) which considered the shortterm metabolic effect of the pattern of food intake on cirrhotic patients. They found that a nibbling pattern of food intake (four small meals compared with two large meals) which included a late evening snack, improved nitrogen balance when compared to the gorging pattern (two large meals per day) but no difference in energy balance was demonstrated. Normally humans consume four to six meals/snacks per day (Langhans 1992) and in the present study there was no significant difference in the number of eating episodes found in control or patient groups.

The mechanisms involved in anorexia that occurs in patients with cirrhosis requires further investigation. Only then will clinically effective and patient acceptable dietary strategies be developed for this patient group.

## OLT Patients

The nutritional management of patients listed for OLT presents clinicians with a dichotomy. In cirrhosis, poor dietary intake may be driven by altered metabolism and negates consumption of a nutritionally adequate diet but following OLT these patients have a propensity toward obesity (Stegall et al 1995; Munoz et al, 1991) and many patients are asked to restrict their energy intake. Weight gain following OLT has not been extensively investigated but it now seems unlikely that this increase in body mass is solely attributable to immunosuppressive drug regimens (Davidson et al, 1998). It could be argued that in the present study the increase in energy and macronutrient intake observed after transplantation is merely a reflection of refeeding mechanisms acting to return the patient's body weight to "set-point" values (Harris, 1990; Dulloo et al, 1996). In the current longitudinal study, fat mass continued to increase even after lean body mass and arm muscle circumference values were comparable to standard values. However, this morphological picture does not parallel refeeding where once the lean body mass is repleted, increases in fat mass stabilise (Dulloo 1997). Nine months after OLT, these patients were 7.5% heavier than their pre-illness "set-point" weight which was reflected in an increase in fat mass.

Whilst no differences in energy intake per kilogram body weight were evident between patients and controls, there was a trend for post-OLT patients to have higher energy intakes. This could exert a cumulative effect on energy intake. For example, six months after transplantation, OLT patients were on average consuming 1.2 MJ/day more than control



subjects (6 months OLT 10.2 MJ/day versus controls 9.0 MJ/day). If extrapolated over a period of a month this would equate to an additional 22 MJ in the OLT group and equates to 0.75 kg of adipose tissue (30 MJ = 1 kg adipose tissue; Mela and Rogers, 1998). This, added to the observed energy economy, would account for a weight gain of 7.5 kg 6 months after transplantation (dietary intake 0.75 kg/month  $\times$  6 = 4.5 kg, energy economy 0.5 kg/month  $\times$  6 = 3.0 kg). Clearly this is an over-simplification of the impact of dietary intake on body weight where activity levels are not considered. Nevertheless, these observed differences in energy intake would contribute to post-OLT weight gain. Interestingly, the magnitude of energy intake at nine months was less than that observed at three and six months after transplantation but this is reflective of the restricted energy diets prescribed to over three-quarters of this OLT population. Transplant recipients appear to be able to restrict their intake of carbohydrate but not fat and this is surprising in that reducing fat intake is the cornerstone of weight reduction dietary programmes.

In post-OLT patients, there was a significant increase at nine months in the energy contribution from dietary fat. In addition, at all study time points the energy derived from fat was greater than that consumed by control subjects where at nine months OLT, the percentage energy derived from fat was 40.9% whereas the contribution was 5% less in control subjects. It could be suggested that the control population were biased where two-thirds worked in a hospital or academic environment and may have been conscious of what constitutes a "healthy diet" (Department of Health, 1992). The most important finding from this



study was the qualitative shift in dietary intake found in those patients studied sequentially where a 5% increase in fat derived energy and a concomitant decrease in carbohydrate derived energy was observed. A particular strength of this longitudinal study is that domestic and environmental factors relating to food preparation were inherently controlled and further underlines the importance of increased consumption of fat derived energy.

The disproportionate increase in dietary fat seen in the current study may be driven by the loss of hepatic innervation and inactivation of hepatic afferent pathways (Friedman, 1988). Following OLT, an absence of hepatic neural signalling to the higher centres could induce hyperphagia and more specifically an increase in fat intake where humoral lipid signalling from CCK and enterostatin, normally acting via hepatic afferents to the brain, will be lost. Paradoxically, following OLT lipid oxidation in both the fasting and fed state was found to be comparable with control subjects but the lack of lipid signalling in OLT patients may result in these patients becoming hyperphagic and more specifically lead to the development of a fat appetite (Sclafani, 1990). Interestingly, Belle et al (1997) in a quality of life study on 346 patients performed one year after OLT, found these patients experienced a significant level of distress due to excess appetite (one of only three distress factors). The OLT time point used in this study would not implicate refeeding mechanisms or drug regimens in the reported 'excess appetite'.

In OLT patients, the proportion of energy derived from dietary fat exceeded recommended intakes after transplantation but this was not so with carbohydrate. However, in these patients glucose gastrointestinal satiety signalling from gastric inhibitory peptide (GIP), glucagon-like peptide (GLP) and insulin remains intact. No difference in the number of eating episodes between control and OLT patients was seen. This suggests that given their higher intakes, that the duration of an eating episode may be prolonged in OLT patients. Furthermore, a diet rich in fat has been shown to extend the intermeal interval (Blundell et al, 1993). This increased consumption of fat observed in liver transplant recipients may be a result of altered macronutrient preference.

## CHAPTER 9

### MACRONUTRIENT PREFERENCE IN LIVER CIRRHOSIS AND IN LIVER TRANSPLANT RECIPIENTS

In the presence of chronic liver disease, it is apparent that bizarre food preferences exist, where the development of cravings for specific foods such as grapefruit have been documented (Deems et al, 1993). In light of reports of aberrant metabolism in chronic liver disease and increased weight following OLT, the development of preferences, not for specific food types but for the macronutrient composition of that food may contribute to these conditions.

The organoleptic properties of fat and carbohydrate mixtures as “real” food is more appropriate in studies which examine macronutrient preference (Drewnowski et al, 1989) rather than methodologies which examine detection and recognition taste thresholds (Smith et al, 1976; Madden et al, 1998) or food frequency questionnaires which provide limited quantitative information (Bingham et al, 1988; Deems et al, 1993).

Nine months following OLT there is a qualitative shift in dietary profile whereby the percentage energy derived from fat is 5% greater than that found in a comparable control group (see Chapter 8). To date no studies have examined macronutrient preference in cirrhotic patients or OLT recipients using mixtures of fat and CHO, the major metabolic fuels.

This study assessed macronutrient preference and the impact of disease severity and primary aetiology in patients with chronic liver

disease. Macronutrient preference was also examined in a sub-group of OLT patients at three, six and nine months following transplantation.

## **Patients and Methods**

### **Patients**

Sixty seven cirrhotic patients (35 BC patients and 32 HC patients) were recruited. Twenty three patients were submitted to OLT and were reviewed on a three monthly basis on three occasions after hospital discharge. Eighteen healthy volunteers were also recruited.

### **Methods**

Preference for fat and CHO was determined using four commercially produced ice creams, referred to as test stimuli. Approximately 15ml of each test stimuli was served in a coded beaker to all subjects (Table 2.1). Hedonic responses were recorded for each sample on a nine point hedonic rating anchored by the responses 'dislike extremely' and 'like extremely'. This methodology is extensively described in Chapter 2.

## **Results**

### **Between group analysis**

The demographic and clinical data of cirrhotic patients and control subjects are fully described in Chapter 3.

## **Macronutrient preference between cirrhotic patients and control subjects**

The control group exhibited a greater preference for the high fat, moderate carbohydrate (HF mod CHO) test stimuli (controls: median = 7.0, IQR = 1.0) than either of the two patient groups (controls versus BC: median = 5.0, IQR = 4.7,  $p < 0.05$ ) (controls versus HC: median = 6.0, IQR = 4.0,  $p < 0.05$ ) (Figure 9.1). No differences in macronutrient preference between the BC and the HC group were apparent.

### **Within group analysis**

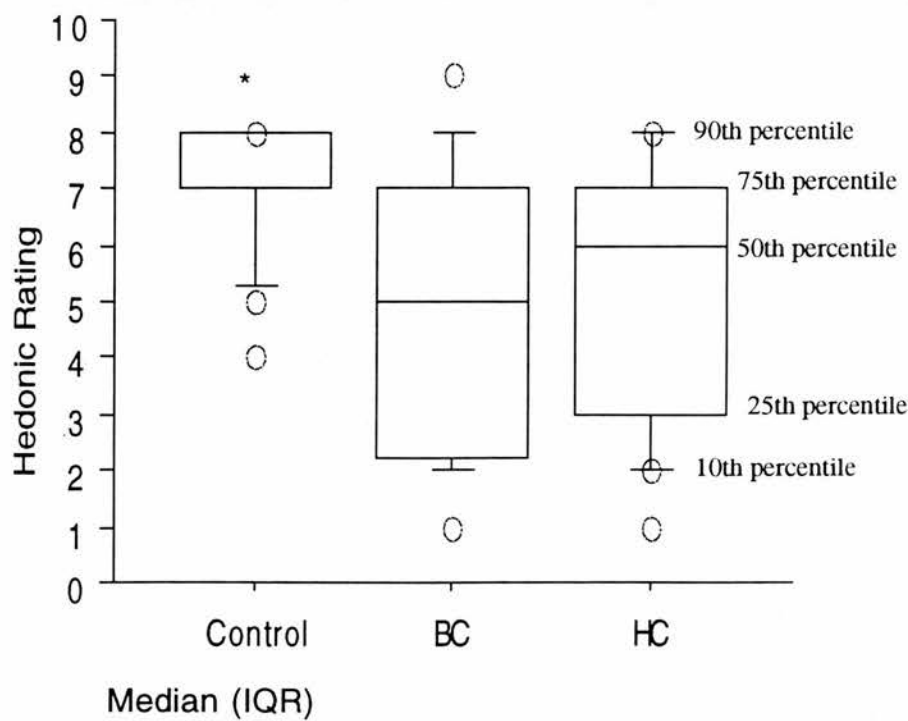
#### **Macronutrient preference within cirrhotic patients and control subjects**

Macronutrient preference was also examined within each group studied. The control group showed no significant preference for any macronutrient combination.

Patients were stratified using Child's Pugh score and no macronutrient preference was apparent within Child's Pugh A, B or C groups. Furthermore, no specific preference was seen when cirrhotic patients were classified by gender.

When patients were stratified by primary aetiology, the BC group demonstrated a greater significant preference for the high fat, high carbohydrate (HF/HCHO) test stimuli (median = 7.0, IQR = 3.0) than the low fat, high carbohydrate (LF/LCHO) test stimuli (median = 5.0, IQR = 4.0,  $p < 0.05$ ). Biliary cirrhotic patients also preferred the HF/ HCHO test stimuli (median = 7.0, IQR = 3.0) to the HF/mod CHO (median = 5.0, IQR = 4.5,  $p < 0.01$ ) test stimuli.

**FIGURE 9.1 PREFERENCE FOR HIGH FAT MODERATE CARBOHYDRATE IN CONTROL SUBJECTS, BILIARY AND HEPATOCELLULAR CIRRHOTIC PATIENTS**



\* =  $p < 0.05$  versus biliary cirrhosis  
\* =  $p < 0.05$  versus hepatocellular cirrhosis

On the other hand, HC patients demonstrated a preference for low fat, moderate CHO (LF/mod CHO) (median = 7.0, IQR = 2.0) test stimuli when compared with HF/mod CHO (median = 6.0, IQR = 4.0,  $p < 0.01$ ). This preference for LF/mod CHO was apparent when compared with the LF/HCHO (median = 6.0, IQR = 3.0,  $p < 0.05$ ) test stimuli. In addition, the HC group preferred HF/HCHO (median = 7.0, IQR = 2.0) to HF/mod CHO ( $p < 0.05$ ) (Figure 9.2) test stimuli.

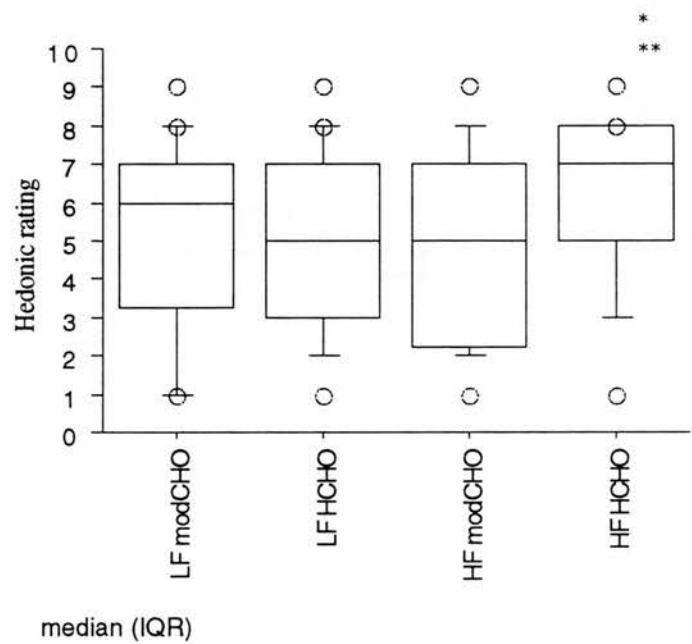
In order to examine the association between fuel oxidation and macronutrient preference the relationship between oxidation rates and hedonic ratings was examined. In the control group a significant association was observed between the LF/mod CHO product and post absorptive rates of glucose oxidation ( $r = 0.58$ ,  $p < 0.05$ ). In other words the higher the rate of glucose oxidation the greater the preference for the food product with the lowest energy content (LF/mod CHO) (Figure 9.3).

In the BC group a significant relationship was observed between the rate of lipid oxidation and preference for the LF/HCHO food ( $r = 0.36$ ,  $p < 0.05$ ). Therefore as the rate of lipid oxidation increases the preference for HF/HCHO increases (Figure 9.3).

In HC patients no relationship between lipid or CHO oxidation rates and macronutrient preference were observed. In addition, no relationship between substrate oxidation rates and dietary intake was seen in any study group. Similarly, no association between dietary intake and macronutrient preference was observed in control subjects or any patient group.

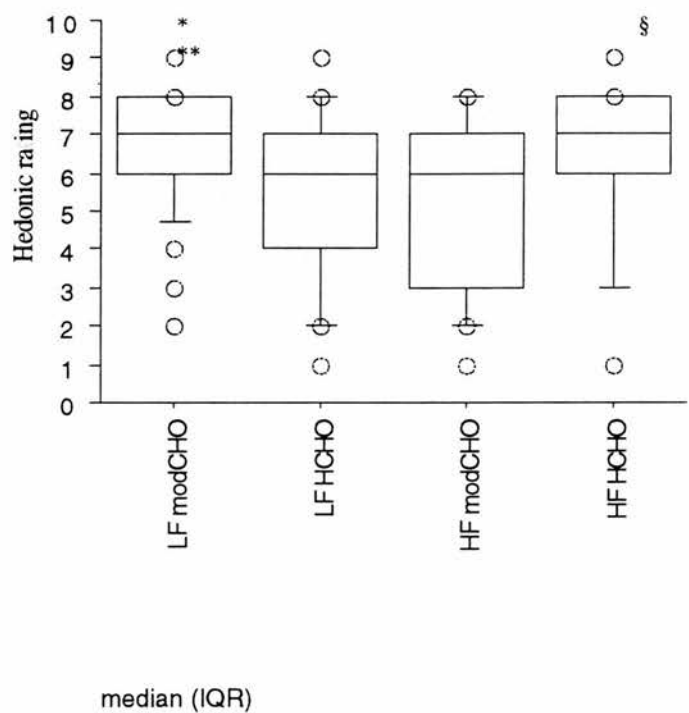
**FIGURE 9.2 MACRONUTRIENT PREFERENCE IN CIRRHOTIC PATIENTS**

**Macronutrient preference in patients with biliary cirrhosis**



\* =  $p < 0.05$ ; preferred to low fat, high carbohydrate  
\*\* =  $p < 0.01$ ; preferred to high fat, moderate carbohydrate

**Macronutrient preference in patients with hepatocellular cirrhosis**

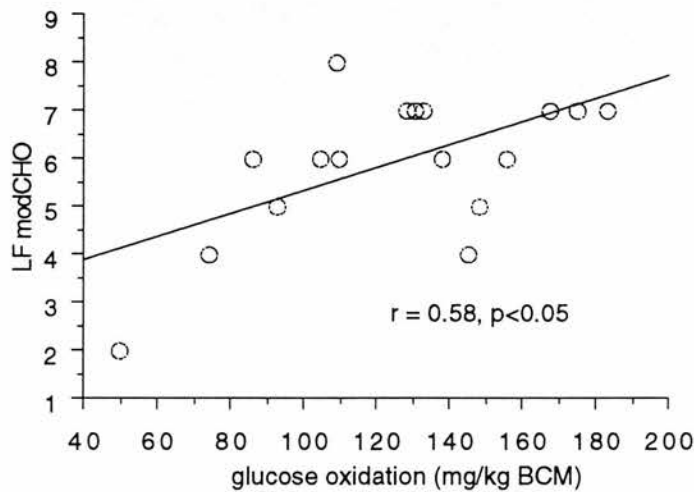


\* =  $p < 0.05$ ; preferred to low fat, high carbohydrate  
\*\* =  $p < 0.01$ ; preferred to high fat, moderate carbohydrate  
§ =  $p < 0.05$ ; preferred to high fat, moderate carbohydrate

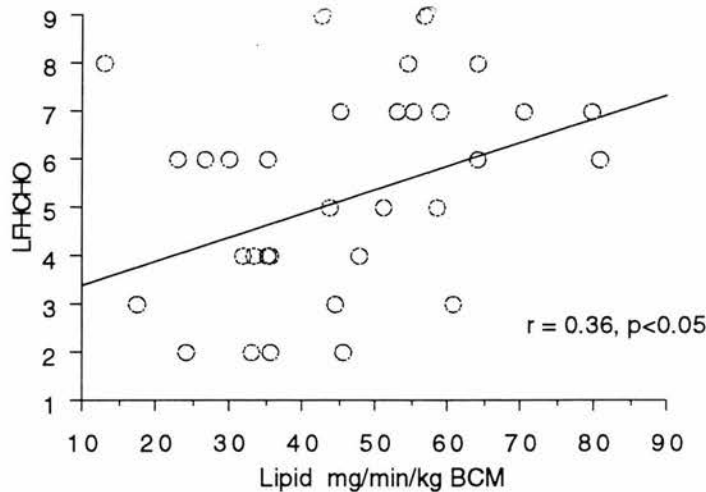


**FIGURE 9.3 ASSOCIATION BETWEEN SUBSTRATE OXIDATION AND TEST STIMULI**

**Association between glucose oxidation and LF mod CHO test stimuli in controls**



**Association between lipid oxidation and LF HCHO test stimuli in biliary cirrhotic patients**



## **OLT Patients**

### **Between group analysis**

Characteristics of OLT patients are shown in Table 3.6.

### **Macronutrient preference between OLT patients and control subjects**

Compared with control subjects the hedonic ratings assigned to each of the four test stimuli were not significantly different from those given by OLT patients at 3, 6 and 9 months after transplantation (Table 9.1). Additionally, no difference in preference was seen when comparisons were made between patients transplanted for BC or HC cirrhosis (Table 9.1).

### **Within group analysis**

#### **Macronutrient preference within OLT patient groups and control subjects**

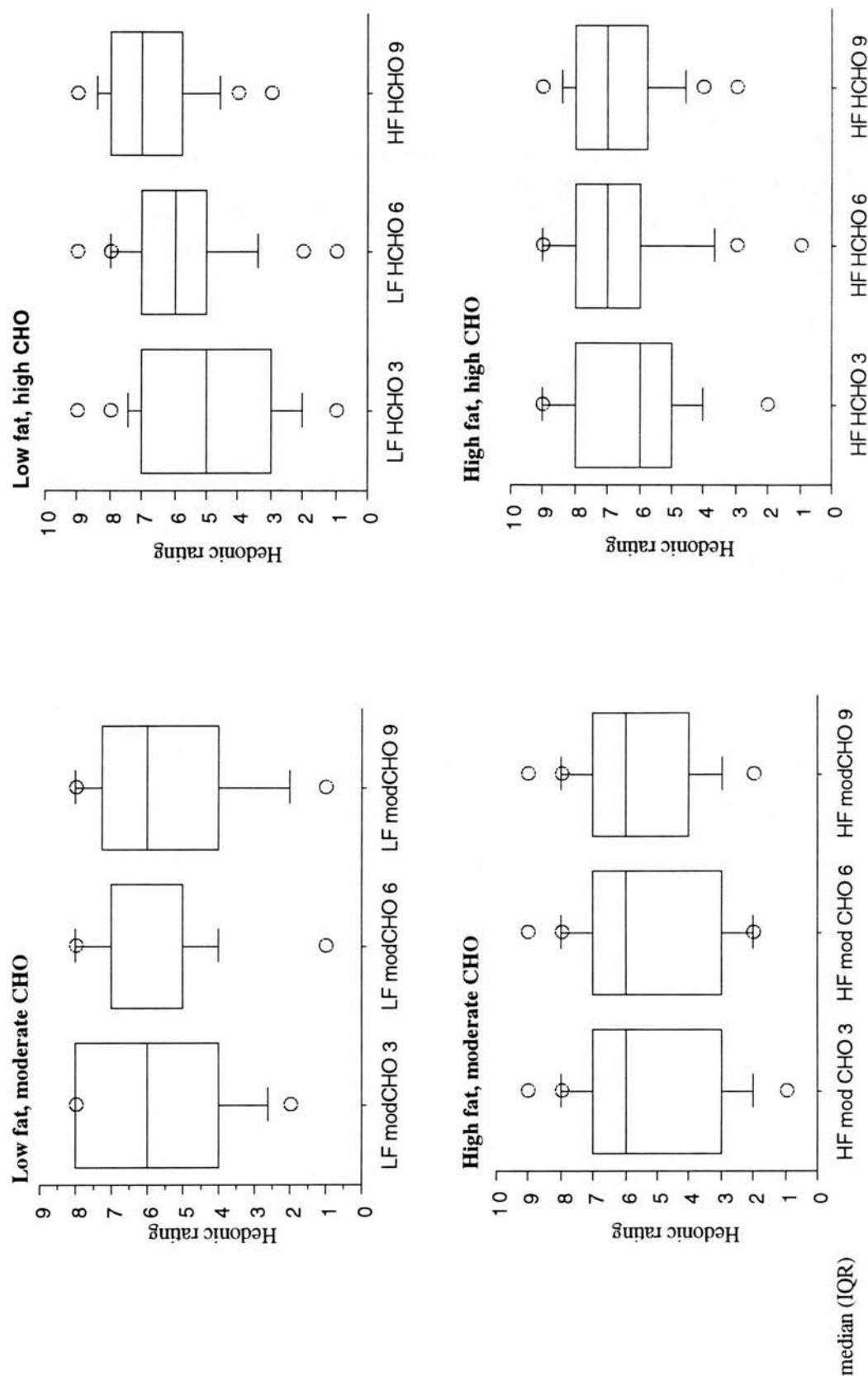
No alteration in preference was observed when test stimuli were examined individually or as a composite group (four test stimuli) at 3, 6 and 9 months OLT (Figures 9.4 and 9.5). In patients transplanted either for BC or HC disease, no difference in preference for individual test stimuli were apparent at any OLT time point. For example, comparison of the hedonic rating for HF/HCHO at 3 months compared with HF/HCHO at 9 months. However, when preference was considered within the group of four test stimuli by nine months OLT those patients transplanted for cholestatic disease exhibited a greater degree of "liking" for the HF/HCHO test stimuli when compared with the LF LCHO test stimuli

**TABLE 9.1 HEDONIC RATINGS FOR TEST STIMULI IN CONTROL SUBJECTS AND PATIENTS FOLLOWING LIVER TRANSPLANTATION**

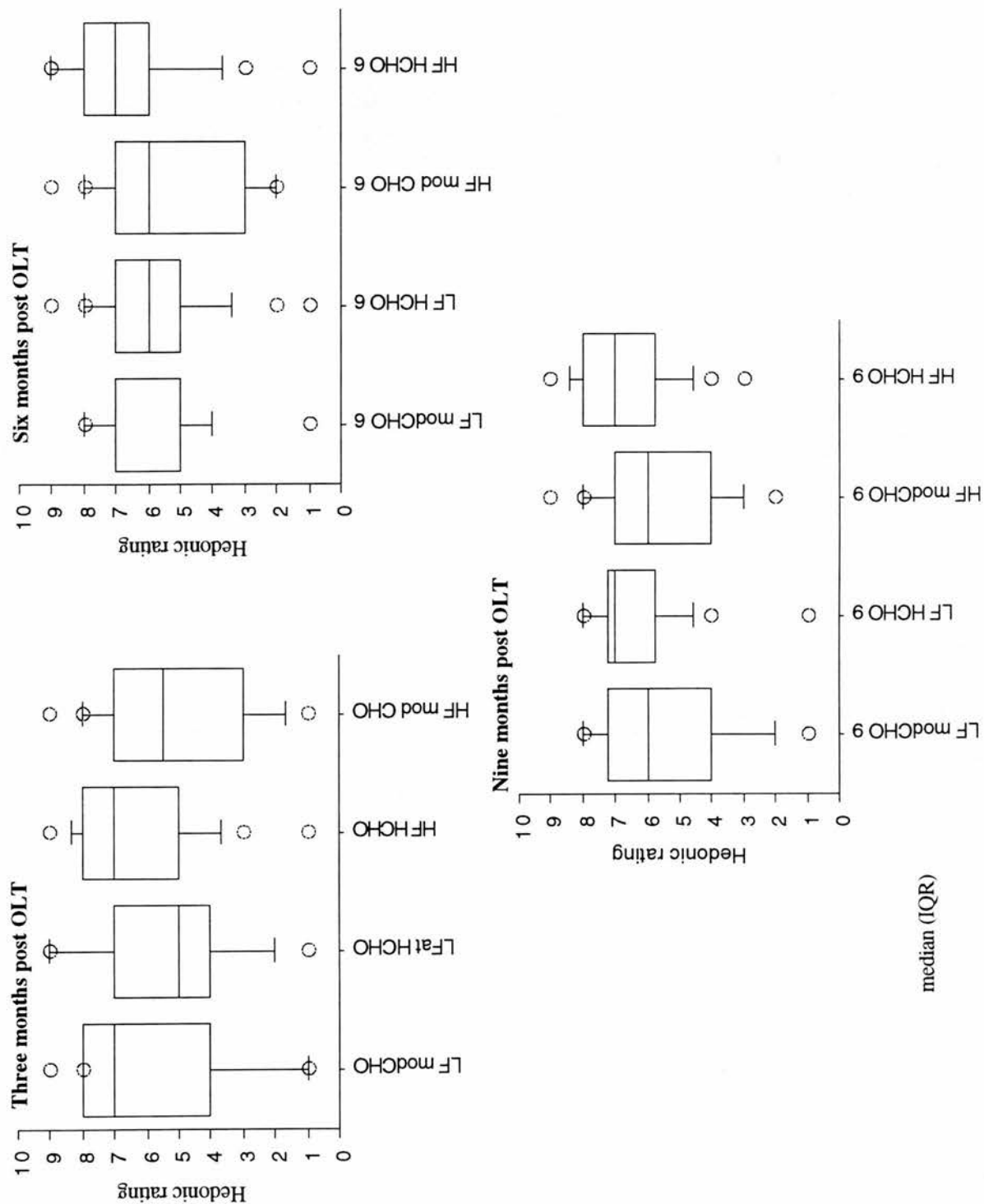
	Low Fat Low CHO	Low Fat Mod CHO	High Fat Mod. CHO	High Fat High CHO
Controls (n = 18)	6.5 (1.0)	6.0 (2.0)	7.0 (1.0)	7.0 (2.0)
3 months OLT (n = 23)	6.0 (4.0)	5.0 (4.0)	6.0 (4.0)	6.0 (3.0)
6 months OLT (n = 23)	7.0 (2.0)	6.0 (2.0)	6.0 (4.0)	7.0 (2.0)
9 months OLT (n = 23)	6.0 (3.0)	7.0 (1.5)	6.0 (3.0)	7.0 (2.2)
3 months OLT BC (n = 23)	7.0 (4.7)	5.0 (4.5)	6.0 (4.0)	6.0 (2.7)
6 months OLT BC (n = 13)	7.0 (2.0)	7.0 (4.0)	4.5 (4.0)	7.5 (4.0)
9 months OLT BC (n = 13)	5.5 (4.0)	6.5 (2.0)	4.0 (3.0)	7.5 (1.5)
3 months OLT HC (n = 10)	5.5 (2.0)	5.5 (2.0)	6.0 (3.0)	6.5 (3.0)
6 months OLT HC (n = 10)	6.5 (2.0)	6.0 (1.0)	6.0 (1.0)	7.0 (2.0)
9 months OLT HC (n = 10)	7.0 (3.2)	7.0 (1.0)	7.0 (2.0)	7.0 (2.2)

median (IQR)

**FIGURE 9. 4 MACRONUTRIENT PREFERENCE FOR EACH TEST STIMULI FOLLOWING LIVER TRANSPLANTATION**



**FIGURE 9.5 MACRONUTRIENT PREFERENCE THREE, SIX AND NINE MONTHS FOLLOWING LIVER TRANSPLANTATION**



(HF/HCHO: 7.5 (IQR = 1.5) versus LF/LCHO: 5.5 (4.0),  $p < 0.05$ ) or the HF/HCHO test stimuli (HF/HCHO = 7.5 (IQR = 1.5) versus HF/modCHO; 4.0 (IQR = 3.0),  $p < 0.05$ ) (Figure 9.6). No specific preferences were seen in patients transplanted for HC disease (Figure 9.7) although there was a general increase in preference ratings (Figure 9.8).

Following OLT no relationship between substrate oxidation rates and macronutrient preference was seen, nor was there any association between preference and dietary intake.

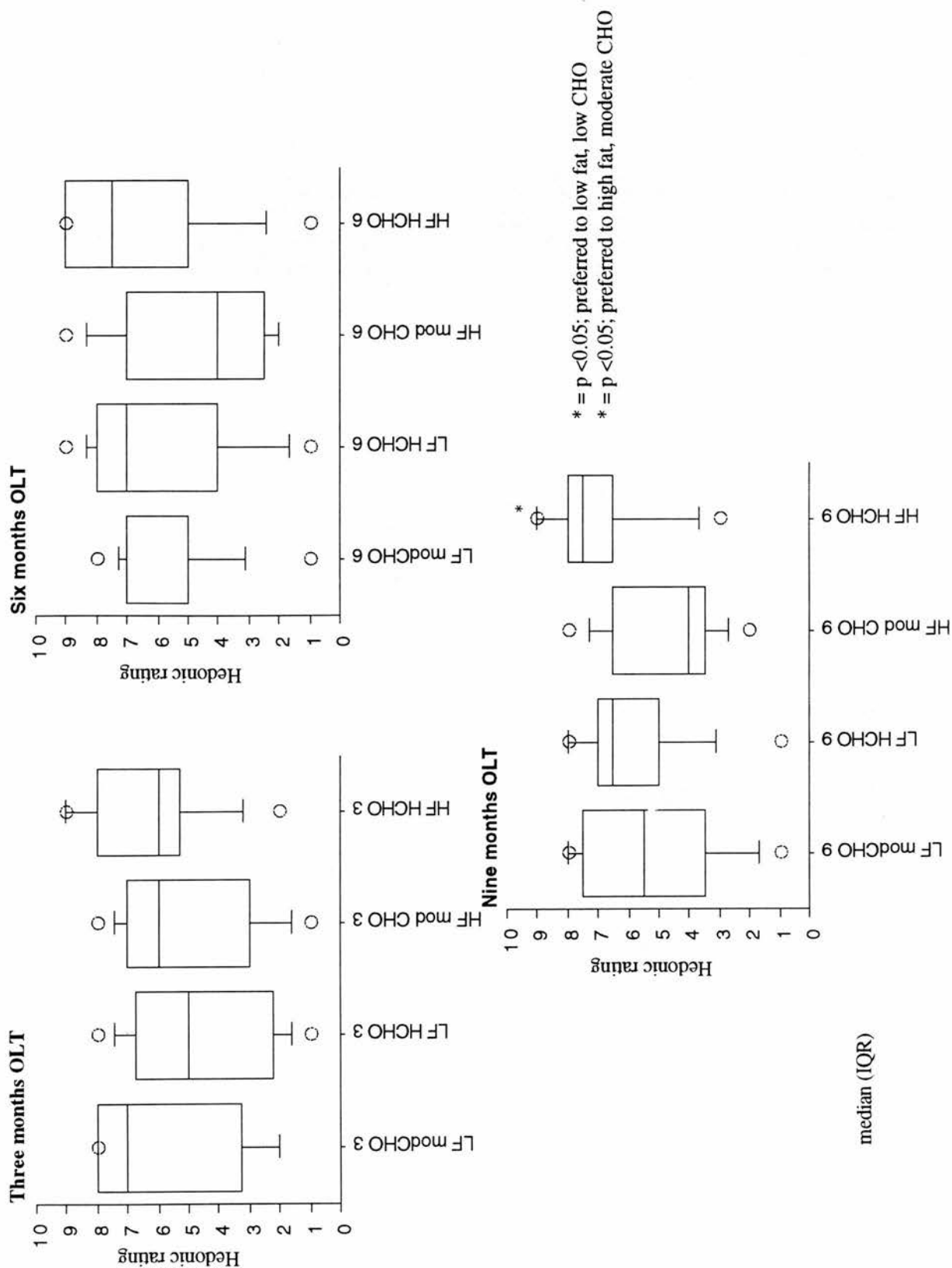
## **Discussion**

### **Cirrhotic patients**

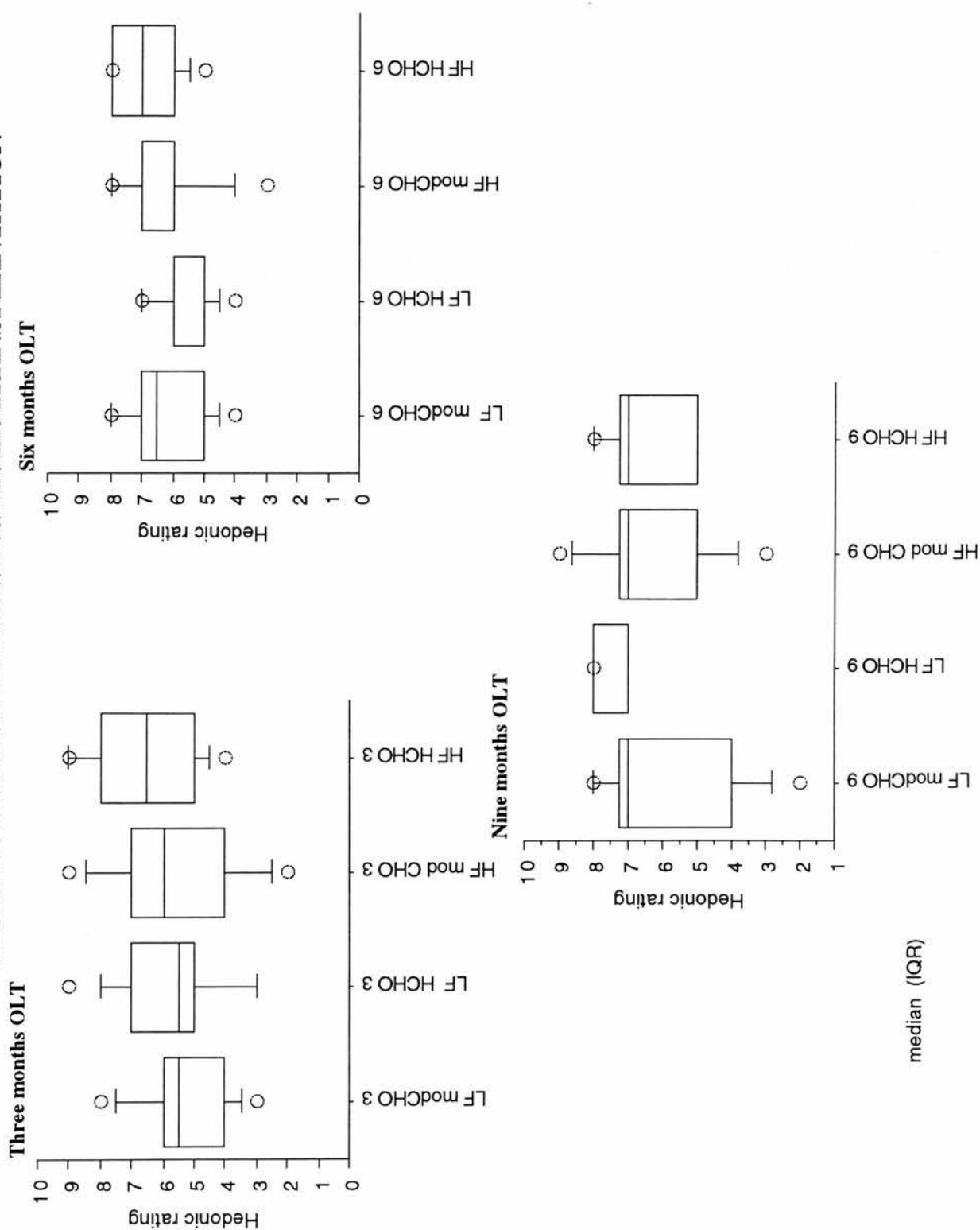
Normally the dietary components, carbohydrate and fat, account for approximately 80% of the energy intake in the United Kingdom population (Department of Health, 1991). Although neither sugar nor fat are consumed individually, it is combinations of these macronutrients that are eaten in foods and at meals. For example, butter is normally consumed along with foods such as bread and jam. The current study adapted methodology traditionally used in food product development or in the study of food preferences, where fat-sugar mixtures based on dairy products (i.e. milk shakes, ice creams), are rated on a nine point hedonic scale.

It has been suggested that animals can learn to associate the sensory properties (palatability) of foods that are metabolically satisfying (Elizalde and Sclafani, 1988). Several studies have shown that changes in physiological state lead to alterations in fat preference and these have

**FIGURE 9.6 MACRONUTRIENT PREFERENCE IN THE BILIARY CIRRHOTIC GROUP FOLLOWING LIVER TRANSPLANT**

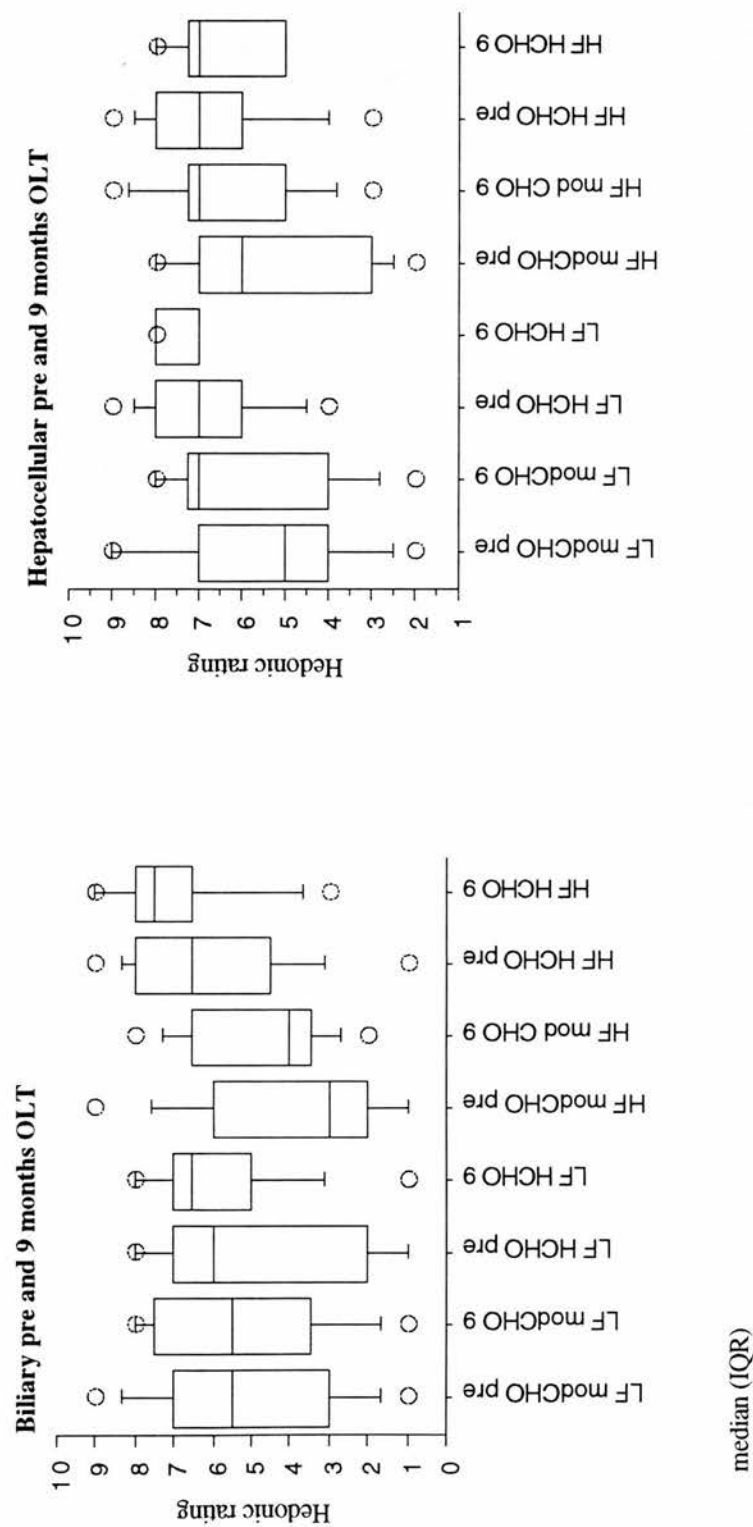


**FIGURE 9.7 MACRONUTRIENT PREFERENCE IN THE HEPATOCELLULAR CIRRHOTIC GROUP FOLLOWING LIVER TRANSPLANTATION**





**FIGURE 9.8 MACRONUTRIENT PREFERENCE IN BILIARY AND HEPATOCELLULAR PATIENTS BEFORE AND AT NINE MONTHS FOLLOWING LIVER TRANSPLANTATION**



shown that with nutrient deprivation and weight loss, there is an increased preference for fat (Reed et al, 1988; Temple et al, 1989; Drewnowski et al, 1992). These studies were performed among healthy individuals and whilst evidence from both animal and human studies confirms that food preference is metabolically driven (Sclafani et al, 1990; Tordoff et al, 1990; Lucas and Sclafani, 1996), consideration of food preference using fat and carbohydrate mixtures has not yet been elucidated in patients with liver disease.

In chronic liver disease, aberrant metabolism may result in altered signaling by afferent pathways to the brain stem, but it is unclear whether this affects the metabolic control of eating including food preferences. In the present study, between group analysis revealed that fasted control subjects exhibited a preference for the HF/modCHO test stimuli when compared with cirrhotic patients but this may simply reflect the highly palatable properties of fat to this healthy population (Mela, 1995).

When macronutrient preference was considered within patient groups, the BC group showed a preference for the HF/HCHO test stimuli. This finding that patients with cholestatic disease exhibited a preference for the high fat food was surprising, as the dietary intake of fat is often prescriptively reduced. However, it may be speculated that the insidious malabsorption of fat in BC could lead to the development of an appetite for high fat foods. The results of the present study are in contrast to those of Deems and Friedman (1988) who reported a reduction in fat intake in bile ligated rats when compared with a control group of animals. However, it should be emphasised that whilst ligation of the common bile

duct will induce cholestasis it does not mimic the slow progressive cholestatic picture found in BC patients.

In the present study, the preference for the HF/HCHO test stimuli evident in BC patients may reflect a drive to consume sufficient energy to maintain endogenous energy reserves (Mela and Rogers, 1998). However, the energy intake of BC patients was significantly lower than control subjects and suggests that in liver disease sensory food preference is not reflected in quantitative intake.

The macronutrient preference was somewhat different in HC patients. A significant preference for the LF/mod CHO test stimuli was observed and only a marginal preference for the HF/HCHO test stimuli when compared with the HF/mod CHO product. The latter finding could be due to the palatability attributes of the HF/HCHO test stimuli which act by masking the CHO content of the food (Drewnowski and Greenwood, 1983) and may not directly be associated with quantitative intake. This is supported by the finding that dietary analysis showed that the CHO and fat intake of the HC group was significantly lower than controls and implies that these patients may consume energy dense foods but only in small quantities. This finding may reflect a physiological inability by HC patients to normally metabolise these energy providing macronutrients and calls into question the effectiveness of the high energy dietary regimen traditionally prescribed for patients with cirrhosis.

The relationship between substrate oxidation and macronutrient selection has been an area of considerable interest in obesity research (Astrup et al, 1996). The present study revealed that an association

between fasting glucose oxidation rate and preference for the LF/LCHO test stimuli in control subjects. This suggests that as the availability of glucose increases this preference for the low energy food product increases but this is not reflected in the dietary intake of these unrestrained eaters. In addition a significant relationship was found between the rate of fat oxidation and preference for LF/HCHO food in BC patients.

The significant relationship between rates of oxidative metabolism and food preference observed in the current study is impressive. After all, substrate oxidation is dictated by the body's requirement to regenerate ATP (Astrup et al, 1996) and it seems likely that in the present study, fasted control subjects would continue to rely on hepatic glycogen reserves as an important and efficient ATP provider. This continued availability of glucose may account for the association between CHO oxidation and the preference for the LF/mod CHO test stimuli.

It is not surprising that no relationship between fasting substrate oxidation and dietary intake was seen as the relationship between these variables is only observed when oxidation rates are determined over a 24 hour period (Thomas et al, 1992). Clearly further studies are required to examine the relationship between substrate utilisation and intake in this group with chronic disease. However, acceptability of the required methodology may prove an obstacle to patient recruitment.

It is well documented that a period of overnight fasting in cirrhotic patients reflects a more prolonged period of fasting (72 hours) (Owen et al, 1982). It should be noted that to date the majority of metabolic studies have been performed on patients with HC disease but work from the

current investigation suggests the response to feeding may be different in patients with BC or HC disease (Richardson et al, 1999). The significant association with lipid oxidation and LF HCHO seen in BC patients may be suggestive of a metabolic drive to minimise lipolysis/gluconeogenesis and thereby preserve fat stores.

In HC patients no association between oxidative metabolism and preference was seen and this may be a consequence of a more disturbed metabolic picture, where for example severe glycogen depletion, enhanced lipolysis and gluconeogenesis may be present. This melee of biological processes which may accompany cirrhosis may serve to distort the relationship between oxidative metabolism and qualitative and quantitative components of ingestive behaviour. The findings of this study suggest that metabolically driven changes in ingestive behaviour are acting to stimulate consumption of a nutritionally inadequate diet.

### **Macronutrient preference following OLT**

Research in the area of macronutrient preference has focused on fat preference and its relationship with obesity (Pangborn et al, 1985; Drewnowski et al, 1987). However, little is known about the central processing of afferent information generated by food that will dictate macronutrient preference. This thesis has underlined the importance of the liver as a metabolic sensor and given that hepatic fuel oxidation can provide unconditioned learning for macronutrient consumption (Tordoff et al, 1990), it would seem feasible that denervation resulting from transplantation may effect changes in food preference and alter central

processing. Following OLT, there is an increase in body mass (Chapter 3) and it may be that alterations in the profile of the macronutrients ingested could contribute to increases in body weight that exceed pre-illness values.

### **OLT patients versus controls**

No differences between control subjects and OLT patients was observed following OLT. This “normalisation” of macronutrient preference was also quantitatively reflected by an increase in OLT patients total energy intake (Chapter 8). In addition, the preferences for HF and LF/modCHO test stimuli exhibited by BC and HC cirrhotic patients respectively were no longer evident three months after transplantation and suggest that pathology specific food preferences have been lost. This is further supported by the fact that differences in dietary intake found in BC and HC patients also disappeared after transplantation (Chapter 8).

### **Macronutrient preference within OLT groups**

Hedonic ratings for individual test stimuli were compared at 3,6 and nine months OLT. In addition, hedonic ratings assigned within the group of four test stimuli presented at each OLT study time point were compared. No macronutrient preference for individual test stimuli or the composite group of four ice creams were evident after OLT. This again suggests that following transplantation, there is a normalisation of macronutrient preference.

Interestingly, when patients were classified by primary pathology, those patients transplanted for cholestatic disease demonstrated a

significant preference for the high fat test stimuli 9 months after transplantation. No significant change in preference was observed within the HC group.

It is difficult to explain the preference for the HF/HCHO test stimuli found within the BC group but not in HC patients. Although, it should be emphasised that the hedonic rating assigned to HF/ HCHO test stimuli by HC patients at nine months OLT was similar to BC patients at nine months. It may be that the discrimination for HF/HCHO within BC patients nine months after OLT is a reflection of the gender bias in this now healthy group. In the BC post-OLT group, there was a predominance of females (1 male : 12 females) and in the HC post-OLT group, there was a predominance of males (9 males : 1 female). However, when macronutrient preference was examined between male and female controls no differences were seen but this could be related the small numbers when patients were sub divided by gender (9 males : 9 females).

Drewnowski and colleagues (1992) in their study on food preferences, revealed specific gender differences and showed that men had a preference for savory sources of fat and CHO, i.e. meat, sausages, hamburgers, margarine/butter whereas women preferred fat/CHO mixtures of ice cream, cakes and cookies. This phenomenon could account for the nine month OLT BC patients greater degree of liking for the HF/HCHO stimuli where their preference for ice cream and the mixture of fat and CHO may be exaggerated in this predominantly female group. However, these differences were not reflected in dietary intake

(Chapter 8) so whilst women prefer “sweeter” fat/CHO mixtures, there appears to be no differential effect on the profile of total energy intake derived from dietary fat.

It is important, however, to emphasise that no differences in preference were seen between BC and HC OLT groups. Indeed there was a general increase in the hedonic ratings assigned to the test stimuli nine months after OLT. This increase in “liking” particularly for the test stimuli high in energy could, if translated into dietary consumption may impact on total energy intake. Interestingly, the preceding chapter has focused on dietary intake and has highlighted the fact that following OLT, there is not only a significant increase in total energy consumption but more importantly the increase in the energy derived from dietary fat which was not only significantly greater than pre-OLT values, but was 5% greater than those found in control subjects.

At present no longitudinal studies in patients in a dynamic nutritional state has systematically tracked individual ratings for macronutrient preference to examine the relationship between preference, dietary intake and body composition. In that respect the current study is unique and shows that the deficit of fat mediated satiety signaling following OLT and the trend to increase hedonic ratings for fat and sugar mixtures may influence dietary consumption. This study has important practical implications where following OLT these patients are routinely given advice on “healthy eating” yet despite the availability of this information, the majority of patients continue to eat a diet rich in fat. It has previously been alluded to that preference for nutritive fat is



enhanced in the presence of food deprivation (Kern et al, 1993). It may be that the lack of fat satiety signaling has deceived central effector mechanisms into believing that the body is in a state of semi-starvation. Clearly further feeding studies that not only consider preference but also the duration of an eating episode will be important to validate this hypothesis.

Despite the complexities of understanding ingestive behaviour, the present study has provided important information that has a practical application. If the reason for high fat consumption is metabolically driven, it seems unlikely that in the short term dietary advice could effect quantitative changes in fat intake. However, given this group's high risk of developing atherosclerosis (Stegall et al, 1995) it may be worthwhile implementing a formal programme of dietary management for these patients. The fat in the diet may be modified to reduce the intake of saturated fats and increase that of mono-unsaturated fats. Clearly the investigation of macronutrient preference gains significance in patients prior to and following OLT with regard to appropriate and achievable dietary advice.

## CHAPTER 10

### THE INFLUENCE OF DRUG REGIMENS ON BODY MASS FOLLOWING LIVER TRANSPLANTATION

Liver transplantation may be a life saving surgical procedure in the management of liver disease but the prolonged survival of patients is dependent upon appropriate immunosuppressive medication. Drugs that prevent rejection of solid organ transplants include prednisolone (Prednisol, Glaxo Laboratories, Middlesex, UK), azathioprine (Imuran Generics Ltd., Hertfordshire, UK), cyclosporin A (Sandimmun, Sandoz Pharmaceuticals, Basel, Switzerland) and tacrolimus (Prograf, Fujisawa Ltd., London). These drugs may be administered as monotherapy or in combination and are associated with metabolic complications such as diabetes, hypertension, hypercholesterolaemia and obesity (Stegall et al, 1995). The influence of immunosuppressive drugs on metabolic complications has been considered in renal (Roth et al, 1989; Esmatjes et al, 1991; Johnston et al, 1993; Tabasco-Minguillan et al, 1993) and liver transplantation (Munoz et al, 1991; Palmer et al, 1991; Stegall et al, 1995).

It would seem logical that administration of comparable immunosuppressive regimens to solid organ transplant recipients would result in a similar prevalence of metabolic complications. However, given that the dose of immunosuppressive therapy administered to liver transplant recipients is substantially less than for renal transplants (Steiger et al, 1995), direct comparisons cannot and have not been made. In

addition the early withdrawal of steroids in OLT has been shown to be a safe treatment modality (Padbury et al, 1993) whereas steroid therapy for renal transplanted patients in most centres continues beyond one year following transplantation. The risk of metabolic complications in OLT may be attenuated by the administration of newer immunosuppressive agents such as tacrolimus (Mor et al, 1995).

Following OLT, the development of obesity may exacerbate any other pre-existing metabolic complications. Furthermore, for the transplant recipient the most visible and often most distressing complication is undesirable weight gain. The fact that the liver transplant recipient has lost all hepatic innervation has ostensibly been ignored and as a result the normal integration of metabolism in higher centres will be lost. This study examined the contribution of OLT immunosuppressive medication, REE and dietary intake on OLT weight change and specific markers of fat mass.

## **Patients and Methods**

### **Patients**

Twenty-three cirrhotic patients were submitted to OLT and had an uncomplicated postoperative recovery. These patients were reviewed on a three monthly basis on three occasions after discharge from hospital. Eighteen healthy volunteers were recruited as controls.

## Methods

Energy expenditure was measured by indirect calorimetry, dietary intake by diet diaries and body composition was assessed using anthropometry and MFBIA. Cumulative immunosuppressive regimens of prednisolone (Prednisol, Glaxo, Middlesex, UK), azathioprine (Imuran, Generics Ltd., Hertfordshire, UK), cyclosporin (Sandimmun, Sandoz Pharmaceuticals, Basel, Switzerland) and tacrolimus (Prograf, Fujisawa Ltd., London) were confirmed from drug prescription charts. Patients with acute cellular rejection were prescribed intravenous hydrocortisone (500 - 1000 mg/day) for three days during their first admission to hospital. Methods are fully described in Chapter 2.

## Results

Patient characteristics are shown in Table 3.7.

Body composition before and following OLT are shown in Table 10.1. Compared with pre-OLT values, there was a significant increase in body habitus measurements at six and nine months after transplantation. A significant increase in lean body mass and a highly significant increase in fat mass and triceps skinfold thickness was seen three to six months after OLT. There was a significant increase in fat mass and triceps skinfold thickness from six to nine months OLT.

Daily immunosuppressive regimens are shown in Table 10.2. No relationship between change in body weight at nine months OLT and corresponding cumulative doses of prednisolone ( $r = 0.03$ , NS), azathioprine ( $r = 0.31$ , NS), cyclosporin ( $r = 0.17$ , NS) and tacrolimus

TABLE 10.1 CHANGES IN BODY COMPOSITION, ENERGY AND FAT INTAKE BEFORE AND FOLLOWING LIVER TRANSPLANTATION

	Pre-OLT	3 Months	6 Months	9 Months	Controls
Body mass index	24.9 (0.90)	25.5 (0.88)	26.9 (0.90) <sup>a,b</sup>	27.8 (0.87) <sup>b,c,h</sup>	26.0 (0.90)
Triceps skinfold (%)	82.5 (7.8)	90.6 (8.6)	115 (7.8) <sup>b,c</sup>	127 (8.1) <sup>b,c,g</sup>	109 (9.2)
Arm muscle circumference (%)	95.1 (3.1)	101.7 (2.4) <sup>a</sup>	104.1 (2.1) <sup>d</sup>	105.3 (1.8) <sup>d,f</sup>	102 (3.3)
Lean body mass (kg)	48.1 (1.9)	48.8 (2.0)	49.1 (2.0) <sup>e,f</sup>	49.8 (2.0) <sup>i</sup>	49.9 (2.2)
Fat mass (kg)	24.5 (1.8)	25.6 (1.3)	28.8 (1.5) <sup>b,c</sup>	30.6 (1.6) <sup>b,c,j</sup>	27.1 (1.5)
Energy intake (MJ/day)	6.40 (0.52)	9.9 (0.81) <sup>d</sup>	10.2 (0.62) <sup>d</sup>	9.4 (0.59) <sup>a</sup>	9.0 (0.45) <sup>j</sup>
Fat intake (g/day)	62.3 (5.4)	106.2 (10.1) <sup>d</sup>	110.4 (7.5) <sup>d</sup>	101.6 (7.1) <sup>d</sup>	83.7 (6.2) <sup>f,i,k</sup>

Mean (±SEM)

a = p<0.01 versus pre-OLT

b = p<0.0001 versus 3 months OLT

c = p<0.0001 versus pre-OLT

d = p<0.001 versus pre-OLT

e = p<0.05 versus pre-OLT

f = p<0.01 versus 3 months OLT

g = p<0.01 versus 6 months OLT

h = p<0.001 versus 6 months OLT

i = p<0.05 versus 3 months OLT

j = p<0.01 versus 6 months OLT

k = p<0.05 versus 9 months OLT

TABLE 10.2 IMMUNOSUPPRESSIVE THERAPY FOLLOWING LIVER TRANSPLANTATION

Time Post-OLT	n	Prednisolone (mg/day)	Azathioprine (mg/day)	Cyclosporin (mg/day)	Tacrolimus (mg/day)
		23	21	12	11
OLT → Discharge (average stay 20.7 ± 1.3)		63.9 ± 15.3	121 ± 31.9	275 ± 20.9	5.4 ± 0.45
3 months post-OLT		11.7 ± 0.97	119 ± 6.9	265 ± 15.0	5.6 ± 0.41
6 months post-OLT		5.4 ± 1.7	106 ± 7.4	280 ± 16.9	5.3 ± 0.36
9 months post-OLT		0.89 ± 0.37	104 ± 7.9	286 ± 21.6	5.6 ± 0.42
Mean (±SEM)					

( $r = 0.11$ , NS) were apparent. No relationship between triple dose immunosuppressive therapy and weight gain following OLT was seen in the 12 patients receiving prednisolone, azathioprine and cyclosporin ( $r = 0.28$ , NS) or the 11 prescribed prednisolone, azathioprine and tacrolimus ( $r = 0.42$ , NS). In addition, there was no association between weight gain and the 11 patients treated with steroids for acute cellular rejection (rejection  $r = 0.093$ , NS; no rejection  $r = 0.27$ , NS). Other factors determined in the present study that may impact on post-OLT weight gain include dietary intake and energy expenditure.

Total energy and fat intake was significantly greater than pre-transplant values. Energy at six months OLT and fat intake at six and nine months OLT was significantly greater than controls.

A significant inverse relationship between fat mass and REE body weight was observed nine months after transplantation (Figure 10.1). Multiple stepwise regression analysis revealed that nine months following liver transplantation, the strongest predictor of increased fat mass was REE (Table 10.3).

## Discussion

Excessive weight gain after kidney transplantation has also been reported (Johnston et al, 1993). Substantive evidence now supports the early withdrawal of steroid therapy following OLT and this has influenced pharmacological practice in the 90's (Padbury et al, 1993; McDiarmid et al, 1995; Gomez et al, 1998). In the SLTU, steroid therapy is normally tapered down and withdrawn three to four months following

FIGURE 10.1

ASSOCIATION BETWEEN REE AND FAT MASS 9 MONTHS FOLLOWING LIVER TRANSPLANTATION

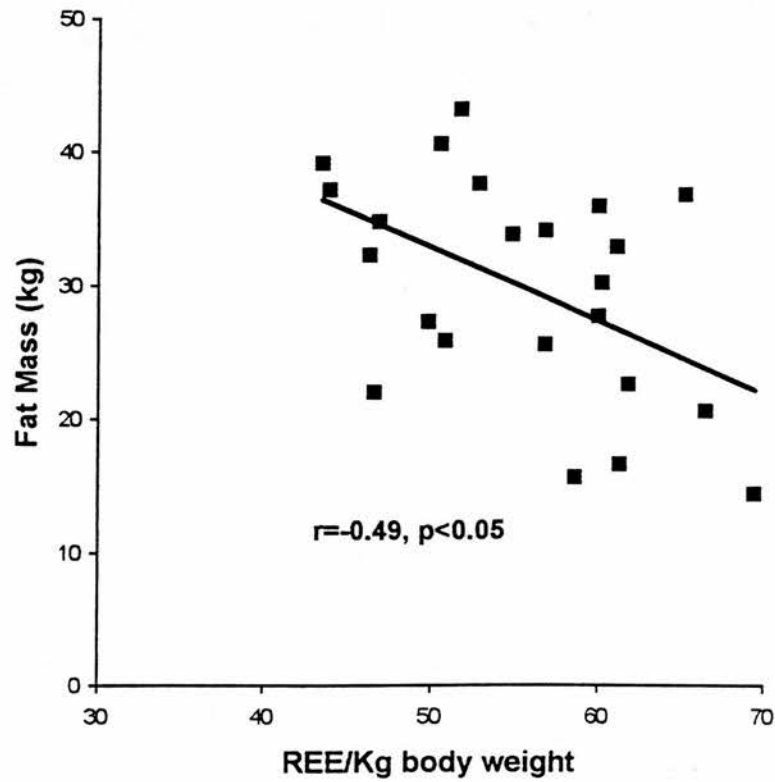




TABLE 10.3 STEPWISE MULTIVARIATE REGRESSION ANALYSIS WITH INDICES OF FAT MASS  
(9 months OLT) AS THE DEPENDENT VARIABLE

Per kg	BMI		TSF (%)		Fat Mass (kg)	
	$\beta$	t	$\beta$	t	$\beta$	t
		p		p		p
REE (kJ)	-0.555	0.001	-0.491	0.028	-0.671	0.001
Fat intake (g)	-0.301	0.165	-0.192	0.405	-0.193	0.324
Prednisolone (mg)	0.050	0.808	0.086	0.690	0.061	0.739
Azathioprine	-0.082	0.689	-0.146	0.492	-0.020	0.913

transplantation. The success rate of early steroid withdrawal in transplantation appears to be more successful in OLT patients compared to other solid organ transplants (heart, lung, kidney) and may be related to the fact that the liver is an immunologically privileged organ (McDiarmid et al, 1995).

Longterm steroid administration produces changes in body mass reflected by an increase in truncal fat deposits, normal peripheral fat content, and a reduction in skeletal muscle mass (Hobber et al, 1986). Although cyclosporin and tacrolimus have been implicated in disturbances in glucose and lipid metabolism (Starzl et al, 1990; Munoz et al, 1991; Stegall et al, 1995), there is no direct evidence to implicate these drugs and the steroid sparing immunosuppressant azathioprine in post-OLT weight gain. An important study by Steiger et al (1995) examined the effect of longterm steroid therapy on body composition up to 16 months after kidney transplantation. This group found no difference in fat mass in females and in males an increase of 0.3 kg fat mass per month was seen. This compares with an increase in body weight of 0.8 kg fat mass per month found in the present study and this increase was seen in both male and female patients (see Chapter 4).

In the present study, no association between the cumulative immunosuppressive medication and body weight was seen when drugs were considered individually or in combination. The lack of association between immunosuppression and weight gain does suggest that the effects of hepatic isolation in terms of autonomic regulatory control may be involved. It is conceivable that the cephalic, post-ingestive, post-

absorptive, humoral and neural responses relayed through hepatic afferents will be lost and may influence feeding behaviour.

The proportion of energy derived from fat in both pre-OLT patients and controls was 35% whereas by six and nine months OLT, this was increased to 40% representing a qualitative shift in dietary profile.

The observed reduction in energy expenditure and increase in energy intake following OLT will result in a positive energy balance and contribute to weight gain. Nine months after OLT, patients' weight exceeded pre-illness values by 7.5 kg or 8% and is surprising given that longterm energy homeostasis is tightly controlled (Harris, 1990). In the present study, the prevalence of overweight and obesity nine months following OLT was found to be about twice that reported in the United Kingdom population (HMSO, 1992).

Multiple regression analysis revealed that the strongest predictor of OLT weight gain is REE. The progressive reduction in REE is difficult to reconcile since these patients could not be classified as hypermetabolic before transplantation. In addition, the presence of healthy rather than diseased hepatocyte should increase REE and the normal response to weight gain is an increase in REE (Leibel et al, 1994). Although activity levels were not objectively assessed in this study, all but one patient returned to normal pre-illness activity levels suggesting there should be an increase in OLT energy expenditure.

Weight gain following liver transplantation cannot be explained on the basis of drug therapy. A recent quality of life study reported that weight gain and increased appetite were causes of distress in OLT patients

(Belle et al, 1997). Unfortunately, for these patients a further restriction on intake may act to exacerbate the apparent energy economy. For these patients, prescribing an energy reduction diet may stimulate feelings of hunger and the drive to eat. As a result, excessive weight gain may be an unavoidable consequence of liver transplantation for the majority of these patients. This represents a challenge for the management of these patients which should include sympathetic dietetic advice combined with an exercise programme. Additionally, for those individuals more susceptible to weight gain, pharmacological intervention may be considered.

## CHAPTER 11

### DISCUSSION AND CONCLUSIONS

Despite considerable interest in the mechanisms involved in undernutrition in chronic liver disease and its impact on outcome, little attention has been paid to the causes of anorexia which will detrimentally affect nutritional status. Therefore, understanding factors that contribute to the reduced spontaneous dietary intake found in cirrhotic patients will prove central in the identification of nutritional regimens acceptable to the patient and at the same time are nutritionally adequate.

The current series of investigations in cirrhotic patients has shown that traditional dietary treatment modalities and prescriptive dietary recommendations for cirrhotic patients are inappropriate, unachievable and as a consequence, unlikely to be clinically effective. It should be noted that all patients studied were in a clinically stable condition and not recovering from an acute or chronic complication of their liver disease. This permitted examination of the effect of cirrhosis *per se* on host metabolism, nutritional intake, macronutrient preference and was not compounded by the physiological responses elicited by a complication such as a variceal bleed or hepatic encephalopathy. These complications may make prescriptive dietary recommendations even more unachievable.

Stratification of cirrhotic patients by primary aetiology has highlighted differences in nutritional status, post-absorptive substrate

oxidation rates, metabolic response to feeding, dietary intake and macronutrient preference. This was not apparent when patients were sub-divided using Child's Pugh classification but is perhaps unsurprising given the dynamic and arbitrary nature of this determinant of disease severity.

Hepatocellular patients' endogenous energy reserves were significantly depleted when compared with either controls or BC patients. The HC patients' post-absorptive lipid oxidation rates were also elevated and reflects an increased reliance on the provision of energy from fat to maintain energy homeostasis. However, on feeding HC patients avidly switched to glucose oxidation. It may be speculated that this is an adaptive response where the reduction in glycogen storage capacity means that glucose must be immediately oxidised to avoid both hyperglycaemia and cellular hypertonicity.

In both the fasted and fed state, plasma insulin concentrations were elevated only in HC patients when compared with controls or BC patients. Insulin secretion is potentiated by CCK, glucagon-like-peptide and gastric inhibitory peptide and are hormones involved in the satiety cascade. Normally in response to feeding these satiety peptides elicit their effects either directly or indirectly through hepatic afferent fibres and bring about cessation of an eating episode. In the present study, the elevated insulin concentrations seen only in HC patients may contribute to early satiety thereby reducing energy intake and impacting on nutritional status. Although not determined in the current study, CCK which is cleared by hepatocytes may be chronically elevated. This

humoral mediator of satiety may act to reduce dietary intake and future metabolic studies should involve measurement of peptides involved in ingestive behaviour.

Energy intakes were found to be lower in BC and HC patient groups when compared with healthy controls. Perhaps more importantly, despite receiving professional advice on consumption of a nutritional intake that meets European guidelines, cirrhotic patients were unable to meet prescribed targets. This energy deficit was most pronounced in HC patients in whom intakes of the energy providing nutrients (CHO and fat) were significantly lower than control subjects. The findings on dietary intake taken in conjunction with insulin responses suggest that the metabolic profile of the cirrhotic patient and in particular in HC disease would negate consumption of a nutritionally adequate diet. This reduced intake may, in part, account for the lower endogenous energy reserves observed in patients with HC disease. In light of these findings, novel strategies that aim to improve the spontaneous nutritional intake of these patients is required and in particular for patients with HC disease who appear to be most at nutritional risk.

It could be assumed that the macronutrient preference studies may go some way toward identifying a nutritional profile that would be acceptable to HC patients. Overall HC patients preferred the low energy food product and this was mirrored in their dietary intake, although HC patients also exhibited a marginal preference for the high fat, high carbohydrate test stimuli. The preference for the low energy product may be a result of the inability of the HC patients to handle and store

glucose. No association between macronutrient preference and substrate oxidation was observed in HC patients who had the most pronounced metabolic disturbances.

Whilst BC patients exhibited a preference for the high fat food product this could be attributed to the development of a fat appetite in a population where insidious malabsorption is a feature of cholestatic cirrhosis. However in BC patients the preference for the high fat product was not directly reflected in intake but it should be emphasised that dietary fat is a nutrient traditionally restricted in cholestatic liver disease. The relationship between lipid oxidation rates and low fat high carbohydrate preference seen in BC patients suggests this may be a metabolic drive to preserve endogenous lipid reserves. In other words as reliance on endogenous lipid increases to meet energy demands, there is a concomitant increase in the preference for low fat, high carbohydrate test stimuli.

The results of macronutrient preference studies suggest that food preference may not directly reflect quantitative intake. For example, whilst BC patients may prefer the high fat food product they may consume only small quantities of these energy dense foods.

The objective in the nutritional management of cirrhotic patients is to sustain a level of nutritional intake that will maintain and improve nutritional status. The results of the present series of investigations suggest that for patients with HC disease the traditional high CHO dietary regimen may only serve to induce early satiety and reduce dietary intake. It may be prudent to improve rather than restrict the fat content of the



diet prescribed to cirrhotic patients and this may be an appropriate way forward in improving dietary intake. However, before universally advocating significant increases in fat, further studies in ingestive behaviour in cirrhotic patients are required. Studies that use pre-loads predominantly containing fat or CHO could be considered and measurement of their effect on subsequent *ad libitum* intake should be measured. Determination of the humoral response to feeding and assessment of appetite and satiety parameters over the time course of the study would also be required. This study of ingestive behaviour may permit identification of the optimal nutritional strategies for cirrhotic patients.

The investigations performed in cirrhotic patients do not unequivocally demonstrate that altered metabolism including aberrant hepatic signalling is responsible for the depressed nutritional intake and status but does strongly suggest that they are contributory. As electrophysiological recordings from the human hepatic afferents are not possible it is difficult to determine the contribution made by hepatic neural input to ingestive behaviour. This also applies to the level of afferent nerve activation caused by pro-inflammatory cytokines.

In summary examination of integrative ingestive behaviour and the complementary biological processes that normally act to maintain energy homeostasis will provide the key to developing clinically effective nutritional regimens for cirrhotic patients. The investigations presented in this thesis have for the first time adopted an integrative approach examining the relationship between energy and substrate metabolism and

ingestive behaviour in cirrhotic patients where primary aetiology appears to exert a differential effect. These findings will have a significant bearing on the future nutritional management of these patients.

Conversely, those patients submitted to OLT and who subsequently have an uncomplicated postoperative course undergo a period of prolonged weight gain. Although this finding is not novel previous investigators have not examined the constituents of post-OLT weight gain or the physiological process that may be involved.

A particular strength of the present study's methodology is that it permitted sequential examination of the effects of OLT on energy and substrate metabolism, nutritional status, spontaneous dietary intake and macronutrient preference as the impact of inter-subject variability is limited (i.e. energy/substrate response, environmental factors in dietary intake).

At surgery, the cirrhotic liver is removed and major blood vessels anastomosed to the donor liver which, however, remains denervated. In other words the cephalic, post-ingestive, post absorptive humoral and neural responses normally relayed through hepatic afferents will be lost and may influence feeding behaviour. Indeed nine months after OLT recipients were shown to have exceeded their reported pre-illness weight by an average of 7.5%, an observation which is not indicative of a simple return to "set-point" body weight. This increase in body mass was reflected not only by an increase in lean tissue but by an appreciably greater accumulation of fat mass.

The prevalence of overweight and obesity in OLT patients was found to be almost twice that found in the United Kingdom population (Department of Health, 1992). The finding that (nine months after OLT) 87% of patients were overweight/obese is of considerable concern given the fragile nature of many of these patients' cardiovascular and bone mineral status. The potential increased risk of OLT morbidity and mortality underlines the significance of identifying mechanisms involved in OLT weight gain.

After transplantation oxidative metabolism, insulin and glucose responses were effectively normalised although a progressive decrease in REE was seen at each OLT study time point. Prior to OLT, patients exhibited no acute phase response and were not hypermetabolic so it is unlikely that this energy economy can be explained by a return to "normal" REE. Furthermore this decrease in energy expenditure was not short lived and continued 9 months after OLT when there would be no residual effects of the surgery. In addition, the majority of OLT recipients returned to a 'normal' level of activity and as a consequence their energy expenditure would increase rather than decrease. In response to feeding, energy expenditure was also significantly lower at the nine month OLT study time point when compared to cirrhotic patients and marginally significantly lower than control subjects.

The progressive energy economy seen in OLT patients may be a consequence of the absence of the integrative control of energy homeostasis. In OLT patients, not only is there a lack of hepatic afferent signalling but also of efferent output and the latter is important in up-

regulating energy metabolism. Following OLT, the metabolic information received by the brain stem would indicate that the host is undergoing a period of nutrient deprivation and initiate a response that decreases energy expenditure in order to preserve body mass. Whilst the exact mechanisms involved in the down-regulation of energy metabolism cannot clearly be defined from the present series of investigations, further studies that consider the role of tri-iodothyronine, leptin and uncoupling proteins in energy metabolism following OLT would confirm or refute the presence of energy economy.

Following transplantation, patients' dietary intake significantly increased but was not surprising given the general improvement in patients' health. However, what was alarming was this was not merely a quantitative effect as there was qualitative shift in the intake of fat derived energy. Nine months after OLT, patients were consuming 5% more energy from fat than control subjects. This occurred despite three-quarters of OLT patients being prescribed low energy (low fat) diets in order to instigate weight loss but patients appeared able to reduce their intake of CHO but not fat.

Following OLT, this increase in total energy and more precisely fat intake may be driven by the loss of hepatic innervation and also the duodenal branch of the vagus which courses with the hepatic vagus. The loss of lipid signalling (CCK, enterostatin and hepatic fuel oxidation) could result in hyperphagia and development of a fat appetite. This is supported by the "distressing" increase in appetite reported in large retrospective studies in OLT patients (at least one year after

transplantation) but not in other groups submitted to solid organ transplantation.

Many clinicians consider weight gain after OLT to be a side effect of immunosuppression and whilst they may contribute to early OLT weight gain, the current studies suggest their effect may have been overstated. Indeed multiple regression analysis revealed that the strongest predictor of OLT weight gain (fat mass, TSF and BMI) is REE followed by dietary fat intake whereas cumulative drug dose was not predictive. These observations underline the influence of the liver in metabolic regulation.

Improvements in donor organ preservation, surgical techniques and immunosuppressive therapy means that the majority of OLT patients look forward to a normal life expectancy. This will be impeded if complications such as hypertension, diabetes, atherosclerosis and osteoporosis develop and will of course be further compounded if recipients are overweight or obese.

There is no doubt patients find this prolonged period of weight gain distressing and become alarmed by a rapid change in their appearance. For example, one subject who had not lost weight prior to OLT (major criteria for transplantation was lethargy), found that by 9 months after hospital discharge her weight had increased by 20 kg and was clearly distressed by the fact that despite reducing her food intake she could not lose weight. Another patient who significantly exceeded their preillness weight (20%) was disturbed when he felt the doctor did not believe he was following the prescribed weight reduction diet. These reports, whilst anecdotal, illustrate the level of anxiety this apparent

inability to lose weight causes OLT patients and is supported by accumulating evidence from quality of life studies that show that weight gain and increased appetite is a negative outcome of OLT. It may be that reducing the dietary intakes of these patients would bring about a further reduction in REE adding to the difficulties these OLT patients face in trying to lose weight. The present study estimates weight gain from OLT energy economy and increased dietary intake accounts for 0.5 kg/month and 1.2 kg/month respectively. Whilst this exceeds the observed weight gain of 0.8 kg/month, no account is made for the energy expenditure due to activity or the non-uniformity of post-transplant weight gain.

This unique study has shown that the effect of drugs on post-OLT weight gain is not as great as first thought and once again, further longitudinal studies on OLT patients are required. These investigations should consider patients responses (metabolic and appetite parameters) to pure macronutrient preloads (protein, fat and carbohydrate) followed by determination of ad libitum intake. Additionally, genetic characteristics of the donor organ to predispose the recipient to obesity has, as yet, not been considered. The use of animal models to pharmacologically characterise receptors (e.g. ATP) on sensory liver afferents will begin to unravel the mechanisms involved in OLT weight gain.

Undertaking the aforementioned studies gain some urgency given the high cardiovascular risk of OLT patients which could be exacerbated in these patients with accelerated weight gain.

## REFERENCES

- Acheson KJ, Schutz Y, Bessard T, Rarussin E, Jequier E. (1984)  
Nutritional influences on lipogenesis and thermogenesis after  
a carbohydrate meal.  
American Journal of Physiology 246: E62-70.
- Achord J.L. (1987)  
Malnutrition and the role of nutritional support in alcoholic  
liver disease.  
American Journal of Gastroenterology 82: 1-7.
- Arase K, Fisler JS, Shargill NS et al. (1988)  
Intraventricular infusions of 3-hydroxybutyrate and insulin in  
a rat model of dietary obesity.  
American Journal of Physiology 255: R974-R981.
- Astrup A, Buemann B, Western P, Toubro S, Raben A,  
Christensen NJ. (1994)  
Obesity as an adaptation to high fat diet: evidence from a  
cross-sectional study.  
American Journal of Clinical Nutrition 59: 350-355.
- Astrup A, Buemann B, Toubro S and Raben A. (1996)  
Defects in substrate oxidation involved in the predisposition  
to obesity.  
Proceedings of the Nutrition Society 55: 817-828.
- Atwater WO, Benedict FG. (1903)  
Experiments on the metabolism of matter and energy in  
the human body.  
USDA Office of Experimental Stations Bulletin Publication,  
No. 136.
- Ballantyne CM, Podet EJ, Patsch WP, Harati Y et al. (1989)  
Effects of cyclosporine therapy on plasma lipoprotein levels.  
Journal of the American Medical Association 262: 53-56.
- Beaton G.H., Milner J, Corey P, McGuire V, Cousins M, Stewart E,  
de Parmos N, Hewitt D, Grambsch PV, Kissim N, Little JA.  
(1979)  
Sources of variance in 24 hour dietary recall data:  
implications for nutrition study design and interpretation.  
American Journal of Clinical Nutrition 32: 2546-2559.
- Belle SH, Porayko MK, Hoofnagle JH, Lake JR, Zetterman RK.  
(1997)  
Changes in quality of life after liver transplantation among  
adults.  
Liver Transplantation and Surgery 3: 93-104.



- Ben-Porat M, Sideman S, Bursztein S. (1983)  
Energy metabolism rate equation for fasting and post-absorptive subjects.  
American Journal of Physiology 244: R764-R769.
- Berkowitz D. (1969)  
Glucose tolerance, free fatty acids, and serum insulin responses in patients with cirrhosis.  
American Journal of Digestive Diseases 14: 691-699.
- Bingham S, Wiggins HS, Englyst H et al (1982)  
Methods and validity of dietary assessments in four Scandinavian populations.  
Nutrition Cancer 4: 23-33.
- Bingham S, Cummings JH. (1985)  
Urine nitrogen as an independent validity measure of dietary intake: a study of nitrogen balance in individuals consuming their normal diet.  
American Journal of Clinical Nutrition 42: 1276-1289.
- Bingham SA, Nelson M, Paul A, Haraldsdottir J, Loken EB, van Staveren WA. (1988)  
Methods for data collection at an individual level.  
In: Manual on Methodology for Food Consumption.  
Eds: Cameron ME and van Staveren WA.  
Pub: Oxford University Press, 53-99.
- Bishop CW, Bowen PE, Ritchley SI. (1981)  
Norms for nutritional assessment of American adults by upper arm anthropometry.  
American Journal of Clinical Nutrition 34: 2530-2539.
- Blundell JE, Burley VJ, Cotton JR, Lawton CL. (1993)  
Dietary fat and the control of energy intake: evaluating the effects of fat on meal size and post-meal satiety.  
American Journal of Clinical Nutrition 57: 772-777.
- Booth RAD, Goodard BA, Paton A. (1966)  
Measurement of fat thickness in man: a comparison of ultrasound, Harpenden calipers and electrical conductivity.  
British Journal of Nutrition 20: 719-725.
- Bosch J, Gomis R, Kravetz D, Casamitjana R, Teres J, Rivera F, Rodes J. (1984)  
Role of spontaneous portal systemic shunting in hyperinsulinism of cirrhosis.  
American Journal of Physiology 247: G206-G212.



- Bruera E, Macmillan K, Kuehn N et al (1990)  
A controlled trial of megestrol acetate on appetite, caloric intake, nutritional status, and other symptoms in patients with advanced cancer.  
*Cancer* 66: 1279-1282.
- Budillon G, Cuomo R, Taccone W, Panico G, Pumpo R, Iaquinto G, Manzillo G. (1996)  
Gastrointestinal peptide hormones in acute viral hepatitis.  
*Italian Journal of Gastroenterology* 28: 86-90.
- Cabre E, Gonzalez-Huiz F, Abad-Lacruz A. et al (1990)  
Effect of total enteral nutrition on the short term outcome of severely malnourished cirrhotics.  
*Gastroenterology* 98: 715-720.
- Campfield LA, Smith FJ, Rosenbaum M, Hirsch J. (1996)  
Human eating: evidence for a physiological basis using a modified paradigm.  
*Neuroscience and Biobehavioral Reviews* 20, 133-137.
- Campillo B, Bovies PN, Deranlay M, Sommer F, Wirquin E, Fouet P. (1992)  
The thermogenic and metabolic effects of food in liver cirrhosis: consequences on the storage of nutrients and the hormonal counter-regulatory response.  
*Metabolism* 41: 476-482.
- Campillo B, Bovies PN, Leluan M. et al (1995)  
Short-term changes in energy metabolism after one month of a regular oral diet in severely malnourished cirrhotic patients.  
*Metabolism* 44: 765.
- Campillo B, Phuong B, Pornun B, Devanlay M. (1997)  
Influence of liver failure, ascites, and energy expenditure on the response to oral nutrition in alcoholic liver cirrhosis.  
*Nutrition* 13: 613-621.
- Caregaro L, Alberino F, Amodio P, Merkel C et al. (1996)  
Malnutrition in alcoholic and virus-related cirrhosis.  
*American Journal of Clinical Nutrition* 63: 602-609.
- Carvallo-Perin P, Cassander M, Bozzo C, Bruno A, Nuccio P, Dall'Omo M, Marucci M, Pagano G. (1985)  
Mechanisms of insulin resistance in liver cirrhosis. Evidence of a combined receptor and post-receptor defect.  
*Journal of Clinical Investigation* 75: 1659-1665.
- Cha K, Chertow GM, Gonzalez J, Lazarus M. Wilmore DW. (1995)  
Multifrequency bioelectrical impedance estimates the distribution of body water.  
*Journal of Applied Physiology* 79: 1316-1319.

- Cohn SH, Parr RM. (1985)  
Nuclear-based techniques for the in vivo study of human body composition.  
Clinical Physics and Physiological Measurement 6: 275-301.
- Crawford DHG, Cuneo RC, Shepherd RW. (1993)  
Pathogenesis and assessment of malnutrition in liver disease.  
Journal of Gastroenterology and Hepatology 8: 89-94.
- Crawford DHG, Shepherd RW, Halliday JW et al. (1994)  
Body composition in nonalcoholic cirrhosis: the effect of disease aetiology and severity on nutritional compartment.  
Gastroenterology 106: 1611-1617.
- Dauncey MJ. (1980)  
Metabolic effects of altering the 24 h energy intake in man using direct and indirect calorimetry.  
British Journal of Nutrition 43: 257-269.
- Davidson HIM, Pattison RM, Richardson RA. (1998)  
Undernutrition and its influence on taste.  
Proceedings of the Nutrition Society 57: 633-638.
- Davidson HIM, Richardson RA, Hinds A, MacGilchrist A, Garden OJ. (1998)  
Changes in body composition following liver transplantation.  
Proceedings of the Nutrition Society 57: 3, 101A.
- Decrock F, Robin JP, Mioskowski E, Herzberg G, Groscolas R, Bach AC. (1998)  
Post-fasting restoration of body lipid and protein in the rat: effect of depletion level.  
International Journal of Obesity 22: (S3), 178.
- Deems RO, Friedman MI. (1988a)  
Macronutrient selection in an animal model of cholestatic liver disease.  
Appetite 11: 73-80.
- Deems RO, Friedman MI. (1988b)  
Altered preferences for sucrose, sodium chloride, urea and hydrochloric acid solutions in an animal model of cholestatic liver disease.  
Physiology and Behaviour 43: 111-114.
- Deems RO, Friedman MI, Friedman LS, Munoz SJ, Maddrey WC. (1993)  
Chemosensory function, food preference and appetite in human liver disease.  
Appetite 20: 209-216.

- Department of Health (1991)  
Dietary Reference Values for Food Energy and Nutrients for the United Kingdom.  
HMSO, London.
- Department of Health (1992)  
Health of a Nation: A Strategy for Health in England.  
HMSO, London.
- Department of Health (1995)  
Obesity. Reversing the Increasing Problem of Obesity in England. Report from the Nutrition and Physical Activity Task Forces.  
Department of Health, London.
- Di Cecco SR, Wieners EJ, Wiesner RH, Southorn PA, Plevak DJ, Rudd AF. (1989)  
Assessment of nutritional status of patients with end-stage liver disease undergoing liver transplantation.  
Mayo Clinic Proceedings 64: 95-102.
- Dolz D, Raurich JM, Ibanez J. et al (1991)  
Ascites increases the resting energy expenditure in liver cirrhosis.  
Gastroenterology 100: 738-744.
- Drewnowski A. (1995)  
Energy intake and sensory properties of food.  
American Journal of Clinical Nutrition 62: 1081S-1085S.
- Drewnowski A, Greenwood MRC. (1983)  
Cream and sugar: human preferences for high fat foods.  
Physiological Behaviour 30: 629-633.
- Drewnowski A, Moskowitz HR. (1985)  
Sensory characteristics of foods: new evaluation techniques.  
American Journal of Clinical Nutrition 42: 924-931.
- Drewnowski A, Halmi KA, Pierce B, Gibbs J, Smith GF. (1987)  
Taste and eating disorders.  
American Journal of Clinical Nutrition 46: 442-450.
- Drewnowski A, Kurth C, Holden-Wiltse J, Saari J. (1992)  
Food preferences in human obesity: carbohydrates versus fats.  
Appetite 18: 207-221.
- Dubois-Dauphin M, Doring KB, Holley A. (1980)  
Topographical relation between the olfactory bulb and the olfactory tract in tench.  
Chem. Senses 5: 159-169.

- Dulloo AG. (1997)  
Human pattern of food intake and fuel-partitioning during weight recovery after starvation: a theory of autoregulation of body composition.  
Proceedings of the Nutrition Society 56: 25-40.
- Dulloo AG, Jacquet J, Girardier L. (1996)  
Autoregulation of body composition during weight recovery in humans: the Minnesota experiment revisited.  
International Journal of Obesity 20: 393-405.
- Durnin J, Womersley J. (1974)  
Body fat assessed from total body density and its estimation from skinfold thickness: measurements on 481 men and women aged from 16-72 years.  
British Journal of Nutrition 32: 77-97.
- Egan J, Mullen KD, Ullman A et al. (1985)  
A prospective evaluation of dietary intake in hospitalised patients with alcoholic liver disease.  
Gastroenterology 88: 1656.
- Elia M. (1992)  
Energy expenditure in the whole body.  
In: Energy Metabolism: Tissue Determinants and Cellular Corollaries.  
Ed: Kinney JM.  
Pub: Raven Press Ltd., New York. pp 19-59.
- Elia M, Livesey G. (1988)  
Theory and validity of indirect calorimetry during net lipid synthesis.  
American Journal of Clinical Nutrition 47: 591-607.
- Elizalde G, Sclafani A. (1988)  
Starch-based conditioned flavour preferences in rats: influence of taste, calories and CS-US delay.  
Appetite 11: 179-200.
- Eriksson LS, Thorne A, Wahren J. (1989)  
Diet-induced thermogenesis in patients with liver disease.  
Clinical Physiology 9: 131-141.
- Esmatjes E. et al (1991)  
Cyclosporine's effect on insulin secretion in patients with kidney transplants.  
Transplantation 52: 500-503.
- EverhartJE, Lombardero M, Lake JR, Weisner RH, Zetterman RK and Hoofnagle J. (1998)  
Weight change and obesity after liver transplantation: Incidence and risk factors.  
Liver Transplantation and Surgery 4: 285-296.

- Fearon KCH, Richardson RA, Hannan WJ, Cowan S, Watson W, Shenkin A, Garden OJ. (1992)  
Bioelectrical impedance analysis in the measurement of the body composition of surgical patients.  
British Journal of Surgery 79: 421-423.
- Flatt JP (1995)  
Body composition, respiratory quotient and weight maintenance.  
American Journal of Clinical Nutrition 62: 1107-1117S.
- Fleisch A. (1951)  
Le metabolisme basal standard et sa determination au moyen du 'metabocalculator'.  
Helvetica Medica Acta 18: 23-27.
- Food Portion Sizes (1994)  
Ministry of Agriculture, Fisheries and Food.  
HMSO, London.
- Forbes GB, Bruining GJ. (1976)  
Urinary creatinine excretion and lean body mass.  
American Journal of Clinical Nutrition 27: 1052-1058.
- Foster KR, Lukaski HC. (1996)  
Whole body impedance - what does it measure?  
American Journal of Clinical Nutrition 64: 388-396.
- Frayn KN. (1983)  
Calculation of substrate oxidation rates in vivo from gaseous exchange.  
Journal of Applied Physiology 55: 628-634.
- Frayn KN. (1997)  
Coping with some extreme conditions.  
In: Metabolic Regulation. A Human Perspective.  
Ed: Snell K.  
Pub: Portland Press, Oxford, pp 163-196.
- Friedman MI. (1988)  
Hepatic nerve function.  
In: The Liver: Biology and Pathobiology (2nd edition)  
Eds: Arias JM, Ja Koby WB, Popper H, Schachter D, Shafritz DA.  
Pub: Raven Press Ltd., New York. pp 949-959.
- Friedman MI. (1995)  
Control of energy intake by energy metabolism.  
American Journal of Clinical Nutrition 62: 1096S-1100S.
- Friedman MI. (1997)  
An energy sensor for control of energy intake.  
Proceedings of the Nutrition Society 56: 41-50.

- Friedman MI, Ramirez I. (1985)  
Relationship of fat metabolism to food intake.  
American Journal of Clinical Nutrition 42: 1093-1098.
- Friedman MI, Sawchenko PE. (1984)  
Evidence for hepatic involvement in control of ad libitum food intake in rats.  
American Journal of Physiology 247: R106-113.
- Friedman MI, Stricker EM. (1976)  
The physiological psychology of hunger: a physiological perspective.  
Psychological Review 83: 409-431.
- Friedman MI, Tordoff MG. (1986)  
Fatty acid oxidation and glucose utilisation interact to control food intake in rats.  
American Journal of Physiology 251: R840-R845.
- Frisancho AR. (1974)  
Triceps skinfold and upper arm muscle size norms for assessment of nutritional status.  
American Journal of Clinical Nutrition 27: 1052-1058.
- Gibson RA. (1990)  
Precision in dietary assessment.  
In: Principles of Nutritional Assessment.  
Ed: Gibson RA.  
Pub: Oxford University Press, pp 97-116.
- Goldberg GR, Black AE, Jebb SA, Cole TJ, Murgatroyd PR, Coward WA, Prentice AM. (1991)  
Critical evaluation of energy intake data using fundamental principles of energy physiology. Derivation of cut-off values to identify under recording.  
European Journal of Clinical Nutrition 45: 569-581.
- Gomez R, Moreno E, Colina F, Loinaz C, Gonzalez-Pinto I, Lumbreras C, Perez-Cerda F, Castellon C, Garcia I. (1998)  
Steroid withdrawal is safe and beneficial in stable cyclosporin-treated liver transplant patients.  
Journal of Hepatology 28: 150-156.
- Greco AVF, Crucitti G, Ghirlanda R, Manna L, Altomonte AG, Rebuzzi AG, Berloli A. (1979)  
Insulin and glucagon concentrations in portal and peripheral veins in patients with hepatic cirrhosis.  
Diabetologia 17: 23-28.
- Greco AV, Mingrone G, Benedetti G, Capristo E. et al (1998)  
Daily energy and substrate metabolism in patients with cirrhosis.  
Hepatology 27: 346-350.

- Green HJ, Bramley PN, Losowsky MS. (1991)  
Are patients with primary biliary cirrhosis hypermetabolic? A comparison between patients before and after liver transplantation and controls.  
Hepatology 14: 464-472.
- Greenblatt DC, Ransil BJ, Harmatz JS et al. (1976)  
Variability of 24 hr urinary creatinine excretion by normal subjects.  
Journal of Clinical Pharmacology 16: 321-328.
- Guglielmi FW, Contento F, Laddaga L, Panella C, Francavilla A. (1991)  
Bioelectric impedance analysis: experience with male patients with cirrhosis.  
Hepatology 13: 892-895.
- Guglielmi FW, Mastronuzzi T, De Marco M et al (1992)  
Oxidative metabolism in cirrhotic patients with and without hepato-cellular carcinoma: effects of malnutrition.  
Hepatology 16: 1144-1149.
- Hannan WJ, Cowen SJ, Fearon KCH, Plester CE, Falconer JS, Richardson RA. (1994)  
Evaluation of multi-frequency bio-impedance analysis for the assessment of extracellular and total body water in surgical patients.  
Clinical Science 86: 479-485.
- Hannan WJ, Cowan SJ, Plester CE, Fearon KCH, De Beaux A. (1995)  
Comparison of bio-impedance spectroscopy and multi-frequency bio-impedance analysis for the assessment of extracellular and total body water in surgical patients.  
Clinical Science 89: 651-658.
- Harris JA, Benedict FG. (1919)  
A biometric study of basal metabolism in man.  
The Carnegie Institute, Washington, 1-266.
- Harris RB. (1990)  
Role of set-point theory in regulation of body weight.  
Federation of American Societies for Experimental Biology 4: 3310-3318.
- Heymsfield SB, Arteaga CL, McManus C, Smith J. (1983)  
Measurement of muscle mass in man: validity of the 24 hour urinary creatinine method.  
American Journal of Clinical Nutrition 37: 478-494.



- Horber FF, Zurcher RM, Herren H, Crivelli MA, Robotti MA, Frey FJ. (1986)  
Altered body fat distribution with glyocorticoid treatment and in patients on long term dialysis.  
American Journal of Clinical Nutrition 43: 758-769.
- Hricik DE, Bartucci MR, Moir EJ, Mayes JT, Schulak JA. (1991)  
Effects of steroid withdrawal on post-transplant diabetes mellitus in cyclosporine-treated renal transplant recipients.  
Transplantation 51: 374-377.
- Jequier E, Acheson K, Schutz Y. (1987)  
Assessment of energy expenditure and fuel utilisation in man.  
Ann Res Nutrition 7: 187-208.
- Jindal RM. (1994)  
Post-transplant diabetes mellitus - a review.  
Transplantation 58: 1289-1298.
- John WJ, Phillips R, Oh L, Adams LJ, McClain CJ. (1989)  
Resting energy expenditure in patients with alcoholic hepatitis.  
Journal of Parenteral and Enteral Nutrition 13: 124-127.
- Johnson CP et al (1993)  
Factors influencing weight gain after renal transplantation.  
Transplantation 56: 822-827.
- Johnson DG, Alberti KGMM, Faber OK et al. (1978)  
C-peptide and insulin in liver disease.  
Diabetes 27: 201-206.
- Johnson SL, McPhee L, Birch LL. (1991)  
Conditioned preferences: young children prefer flavours associated with high dietary fat.  
Physiology and Behaviour 50: 1245-1251.
- Jhangiani SS, Agarwal N, Holmes R, Caylon CG, Pitchumoni CS. (1986)  
Energy expenditure in chronic alcoholics with and without liver disease.  
American Journal of Clinical Nutrition 44: 323-329.
- Kabadi UM, Eistenstein AB, Tucci J, Pellicore J. (1984)  
Hyperglucagonemia in hepatic cirrhosis: its relation to hepatocellular dysfunction and normalization on recovery.  
American Journal of Gastroenterology 79: 143-149.
- Kaplan MM. (1987)  
Primary biliary cirrhosis.  
Adv. Intern. Medicine 32: 359-378.



- Kasiske BL (1988)  
Risk factors for accelerated atherosclerosis in renal transplant recipients.  
*American Journal of Medicine* 84: 985-992.
- Kasiske BL, Guijarro C, Massy ZA, Wiederkehr MR, Ma JZ. (1996)  
Cardiovascular disease after renal transplantation.  
*Journal of the American Society of Nephrology* 7: 158-165.
- Kern DL, McPhee L, Fisher J, Johnston S, Birch LL. (1993)  
The post-ingestive consequences of fat condition preferences for flavours associated with high dietary fat.  
*Physiology and Behaviour* 54: 71-76.
- Keys A, Brozek J, Henschel A, Michelsen O, Taylor HL. (1950)  
The biology of human starvation, volume 2.  
Minneapolis: University of Minnesota Press.
- Kinney JM, Morgan AP, Domingues FJ, Gildner KJA. (1964)  
A method for simultaneous measurement of gas exchange and expired radioactivity in acutely ill patients.  
*Metabolism* 13: 205-211.
- Kleiber M. (1932)  
Body size and metabolism.  
*Hilgardia* 6: 315-353.
- Klipstein-Grobusch K, Kroke A, Boeing H. (1998)  
Reproducibility of self-reported past body weight.  
*European Journal of Clinical Nutrition* 52, 525-528.
- Kondrup J, Muller MJ. (1997)  
Energy and protein requirements of patients with chronic liver disease.  
*Journal of Hepatology* 27: 239-247.
- Kruszynska YT, Williams N, Perry M, Herne P. (1988)  
The relationship between insulin sensitivity and skeletal muscle enzyme activities in liver cirrhosis.  
*Hepatology* 8: 1615-1619.
- Kruszynska YT, Herne PD, McIntyre N. (1991)  
Relationship between insulin sensitivity, insulin secretion and glucose tolerance in cirrhosis.  
*Hepatology* 14: 103-111.
- Kruszynska YT, Meyer-Alber A, Darakhshan F, Horne PD, McIntyre N. (1993)  
Metabolic handling of orally administered glucose in cirrhosis.  
*Journal of Clinical Investigation* 91: 1057-1066.

- Kushner RF, Schoeller DA. (1986)  
Estimate of total body water by bioelectrical impedance analysis.  
American Journal of Clinical Nutrition 41: 810-817.
- Lammert O, Garby L, Maron K. et al (1987)  
Effect of the preceding day's energy intake on the energy cost of rest, arm and leg exercise.  
Human Nutrition Clinical Nutrition 41C: 141-147.
- Langhans W. (1991)  
Hepatic and intestinal handling of metabolites during feeding in rats.  
Physiol. Behav. 49: 1203-1209.
- Langhans W. (1996)  
Role of the liver in the metabolic control of eating: what we know and what we do not know.  
Neuroscience Biobehavioural Review 20: 145-153.
- Langhans W, Scharrer E. (1992)  
Metabolic control of eating.  
World Review of Nutrition and Dietetics.  
ed. Simopoulos AP, Basel, 70: 1-67.
- Larson DE, Ferraro RT, Robertson DS, Ravussin E. (1995)  
Energy metabolism in weight-stable post-obese individuals.  
American Journal of Clinical Nutrition 62: 735-739.
- Lautz HU, Selberg O, Korberg J. et al (1992)  
Protein-calorie malnutrition in liver cirrhosis.  
Clin. Invest. 70: 478.
- Leibel RL, Rosenbaum M, Hirsch J. (1995)  
Changes in energy expenditure resulting from altered body weight.  
New England Journal of Medicine 332: 621-628.
- Levine JA, Stanger LC, Morgan MY. (1989)  
Resting and post-prandial energy expenditure and fuel utilisation in patients with compensated cirrhosis and control subjects.  
Clinical Nutrition 11: 54 (abstract).
- Lucas F, Sclafani A. (1996)  
Food deprivation increases the rat's preference for a fatty flavour over a sweet taste.  
Chemical Senses 21: 169-179.
- Lukaski H. (1986)  
Use of the tetrapolar bioelectrical impedance method: a validation study.  
Journal of Applied Physiology 60: 1327-1332.

- Lukaski H. (1996)  
 Estimation of muscle mass.  
 In: Human Body Composition.  
 Eds. Roche AF, Heymsfield SB, Lohman TG.  
 Pub: Human Kinetics, Leeds, UK. pp 109-125.
- Lykken GI, Jacob RA, Munoz JN, Sandstead HH. (1980)  
 A mathematical model of creatine metabolism in normal males: comparison between theory and experiment.  
 American Journal of Clinical Nutrition 33: 2674-2685.
- MacDiarmid JI, Blundell JE. (1997)  
 Dietary under-reporting: what people say about recording their food intake.  
 European Journal of Clinical Nutrition 51: 199-200.
- McDiarmid S, Farmer D, Goldstein L, Martin P, Vargus J, Tiplon J. (1995)  
 A randomised prospective trial of steroid withdrawal after liver transplantation.  
 Transplantation 60: 1443-1450.
- McMaster P. (1994)  
 The development of liver transplantation.  
 In: Liver Transplantation, Practice and Management.  
 Eds: Neuberger J, Lucey MR.  
 Pub: BMJ Publishing Group, London. pp 3-7.
- Madden A, Bradbury W, Morgan M. (1997)  
 Taste perception in cirrhosis: its relationship to circulating macronutrients and food preferences.  
 Hepatology 26: 40-48.
- Mayer J. (1955)  
 Regulation of energy intake and the body weight: the glucostatic theory and lipostatic hypothesis.  
 Annals of the New York Academic of Sciences 63: 15-42.
- Megyesi C, Samols E, Marks V. (1967)  
 Glucose intolerance and diabetes in chronic liver disease.  
 Lancet ii, 1051-1055.
- Mela DJ. (1995)  
 Understanding fat preferences and consumption: applications of behavioural sciences to a nutritional problem.  
 Proceedings of the Nutrition Society 54: 453-464.
- Mela DJ, Rogers PJ. (1998)  
 Control of appetite and energy balance and imbalance.  
 In: Food Energy and Obesity.  
 Eds: Mela DJ, Rogers PJ.  
 Pub: Chapman and Hall, London. pp 1-13.

- Mendenhall CL, Tosch T, Weesner RE, Garcia-Pont P et al. (1986)  
VA cooperative study on alcoholic hepatitis II: prognostic significance of protein-calorie malnutrition.  
*American Journal of Clinical Nutrition* 43: 213-218.
- Mendenhall CL, Moritz TE, Roselle GA, Morgan TR et al. (1995)  
Protein energy malnutrition in severe alcoholic hepatitis: diagnosis and response to treatment.  
*Journal of Parenteral and Enteral Nutrition* 19: 258-265.
- Merion RM, Twork AM, Rosenberg L, Ham J, Burtch GD, Turcotte JG, Rocher LL, Campbell DA. (1991)  
Obesity and renal transplantation.  
*Surgery, Gynecology & Obstetrics* 172: 367-376.
- Merli M, Riggio O, Dally L. (1996)  
Does malnutrition affect survival in cirrhosis?  
*Hepatology* 23: 1041-1046.
- Merli M, Riggio O, Romiti A, Anosto F, Mango L, Pinto G et al (1990)  
Basal energy production rate and substrate use in stable cirrhotic patients.  
*Hepatology* 12: 106-112.
- Mills PR, Shenkin A, Anthony RS et al. (1983)  
Assessment of nutritional status and in vivo immune responses in alcoholic liver disease.  
*American Journal of Clinical Nutrition* 38: 849-859.
- Ministry of Agriculture, Fisheries and Food (MAFF) (1993)  
Food Portion Sizes.  
HMSO, London.
- Moore FD. (1980)  
Energy and the maintenance of the body cell mass.  
*Journal of Parenteral and Enteral Nutrition* 4: 228-260.
- Mor E, Facklam D, Fasse J, Sheiner P, Emre S, Schwartz M, Miller C. (1995)  
Weight gain and lipid profile changes in liver transplant recipients: long term results of the American FK506 multicenter study.  
*Transplantation Proceedings* 27: 1126.
- Moreau R, Lee SS, Soupison T. (1988)  
Abnormal tissue oxygenation in patients with cirrhosis and liver failure.  
*Journal of Hepatology* 7: 98-105.
- Morgan MY. (1982)  
Alcohol and nutrition.  
*British Medical Bulletin* 38: 21-29.

- Müller MJ, Böker KHW, Selberg O. (1994)  
Are patients with liver cirrhosis hypermetabolic?  
Clinical Nutrition 13: 131-144.
- Müller MJ, Fenk A, Lautz HU, Selberg O, Canzler H, Balks HJ et al. (1991)  
Energy expenditure and substrate metabolism in ethanol-induced liver cirrhosis.  
American Journal of Physiology 260: E338-E344.
- Müller MJ, Lautz HU, Plogmann B. et al (1992a)  
Energy expenditure and substrate oxidation in patients with cirrhosis: the impact of cause, clinical staging and nutritional state.  
Hepatology 155: 782-793.
- Müller MJ, Willmann O, Rieger A. et al (1992b)  
Mechanisms of insulin resistance associated with liver cirrhosis.  
Gastroenterology 102: 2033-2041.
- Müller MJ, Loyal S, Schwarze M. Lobers J. Selberg O, Ringe B, Pichlmayr R. (1994)  
Resting energy expenditure and nutritional state in patients with liver cirrhosis before and after liver transplantation.  
Clinical Nutrition 13: 145-152.
- Munoz SJ, Deems RO et al. (1991)  
Hyperlipidemia and obesity after orthotopic liver transplantation.  
Transplantation Proceedings 23: 1480-1483.
- Navasa M, Bustamante J, Marroni C, Gonzalez E, Andreu H, Esmatjes E et al. (1996)  
Diabetes mellitus after liver transplantation: prevalence and predictive factors.  
Journal of Hepatology 25: 64-71.
- Nettleton PA, Day KC, Nelson N. (1980)  
Dietary survey methods. A comparison of nutrient intakes within families assessed by household measures and the sernweighed method.  
Journal of Human Nutrition 34: 349-354.
- Nielsen K, Kondrup J, Martinsen L, Stilling B, Wikman B. (1993)  
Nutritional assessment and adequacy of dietary intake in hospitalised patients with alcoholic liver cirrhosis.  
British Medical Journal 69: 665-679.

- Nielsen K, Kondrup J, Martinsen L, Dossing H, Stilling B, Jensen MG. (1995)  
Long-term oral refeeding of patients with cirrhosis of the liver.  
British Journal of Nutrition 74: 665-679.
- Niiijima A. (1982)  
Afferent impulse discharges from glucoreceptors in the liver of the guinea pig.  
Annals of the New York Academy of Sciences 157: 690-700.
- Nolte W, Hartmann H, Ramadori G. (1995)  
Glucose metabolism and liver cirrhosis.  
Exp. Clinical Endocrinology 103: 63-74.
- Nosadini R, Arogaro A, Mollo F, Marescotti C, Tiengo A, Duner E, Merkel C. et al (1984)  
Carbohydrate and lipid metabolism in cirrhosis: evidence that hepatic uptake of gluconeogenic precursors and of free fatty acids depends on effective hepatic flow.  
Journal of Clinical Endocrinology and Metabolism 58: 1125-1132.
- Novin D, Robinson K, Culbreth LA, Tordoff MG (1985)  
Is there a role for the liver in the control of food intake.  
American Journal of Clinical Nutrition 42: 1050-1062.
- Ohyanagi H, Nomura H, Nischimatsu S, Usami M, Kosahara H. (1995)  
The liver and nutrient metabolism.  
In: Artificial Nutrition Support in Practice.  
Eds: Payne-James J, Grimble G, Silk D.  
Pub: Edward Arnold, London. pp 59-71.
- Orrego H, Blake JE, Blendis LM, Compton KV, Israel Y. (1987)  
Long term treatment of alcoholic liver disease with propylthiouracil.  
New England Journal of Medicine 317: 1421-1426.
- Ost L. (1984)  
Effects of cyclosporine on prednisolone metabolism.  
Lancet 25: 451.
- Owen OE, Reichle FA, Mozzoli MA et al (1981)  
Hepatic, gut and renal substrate flux rates in patients with hepatic cirrhosis.  
Journal of Clinical Investigation 68: 240-252.
- Owen OE, Trapp VE, Reichard GA, Mozzoli MA, Moctezuma J, Paul P. et al (1983)  
Nature and quantity of fuels consumed in patients with alcoholic cirrhosis.  
Journal of Clinical Investigation 72: 1821-1832.

- Padbury R, Gunson B, Dousset B, Hubscher S, Buckels J, Neuberger J, Elias E, McMaster P. (1993)  
Steroid withdrawal from long term immunosuppression in liver allograft recipients.  
Transplantation 55: 789-794.
- Palmer M, Schaffner F, Thung SN. (1991)  
Excessive weight gain after liver transplantation.  
Transplantation 51: 797-800.
- Pangborn RM, Bos KEO, Stern JS. (1985)  
Dietary fat intake and taste responses to fat in milk by under, normal and overweight women.  
Appetite 6: 25-40.
- Pekkarinen M. (1970)  
Methodology in the collection of food consumption data.  
World Review Nutrition and Dietetics 12: 145-171.
- Peryam DR, Pilgrim FJ. (1957)  
Hedonic scale method of measuring food preference.  
Food Technology 11: 9-14.
- Petrides AS, De Fronzo RA. (1989)  
Glucose and insulin metabolism in cirrhosis.  
Journal of Hepatology 8: 107-114.
- Petrides AS, Vogt C, Schulze-Berge D, Matthews DE, Strohmeyer G. (1994)  
Pathogenesis of glucose intolerance and diabetes mellitus in cirrhosis.  
Hepatology 19: 616-627.
- Pett LB, Ogilvie GF. (1956)  
The Canadian weight-height survey.  
Human Biology 28: 177-188.
- Pirsch JD, Armbrust MJ, Knechtle SJ, D'Alessandro AM, Sollinger HW, Heisey DM, Belzer FO. (1995)  
Obesity as a risk factor following renal transplantation.  
Transplantation 59: 631-647.
- Plauth M, Merli M, Kondrup J, Weimann A, Ferenci P, Muller MJ. (1997)  
ESPEN Guidelines for Nutrition in Liver Disease and Transplantation.  
Clinical Nutrition 16: 43-55.
- Poppitt SD, Swann D, Black AE, Prentice AM. (1998)  
Assessment of selective under-reporting of food intake by both obese and non-obese women in a metabolic facility.  
International Journal of Obesity 22: 303-311.



- Posner BI. (1987)  
Insulin interaction with the central nervous system: nature and possible significance.  
Proceedings of the Nutrition Society 46: 97-103.
- Prijatmoko D, Strauss BJG, Lambert JR, Sievert W. et al (1993)  
Early detection of protein depletion in alcoholic cirrhosis: role of body composition analysis.  
Gastroenterology 5: 1839-1845.
- Pugh RNH, Murray-Lyon IM, Dawson JL, Pietroni MC, Williams R. (1973)  
Transection of the oesophagus for bleeding oesophageal varices.  
British Journal of Surgery 60: 646-649.
- Punch JD, Shieck VL, Campbell DA, Bromberg JS, Turcotte JG, Merion RM. (1995)  
Corticosteroid withdrawal after liver transplantation.  
Surgery 118: 783-786.
- Raben A, Andersen HB, Christensen NJ, Madsen J, Holst JJ, Astrup A. (1994)  
Evidence for an abnormal postprandial response to a high-fat meal in women predisposed to obesity.  
American Journal of Physiology 267: E549-E559.
- Ravussin E, Bogardus C. (1989)  
Relationship of genetics, age and physical fitness to daily energy expenditure and fuel utilisation.  
American Journal of Clinical Nutrition 49, 968-975.
- Reed DR, Contreras RC, Maggio C, Greenwood MRC, Rodin J. (1988)  
Weight cycling in female rats increases dietary fat selection and adiposity.  
Physiology and Behaviour 42: 389-395.
- Rennie M. (1995)  
Protein and amino acid metabolism in the whole body and in the tissues.  
In: Artificial Nutrition Support in Clinical Practice. Eds. Payne-James J, Grimble G, Silk D. 1st Edition. Pub: Edward Arnold, London. pp 13-41.
- Richardson RA, Davidson HIM, Garden OJ. (1998)  
The impact of biliary and hepatocellular cirrhosis on nutritional intake and macronutrient preference.  
Clinical Nutrition 17: 18.



- Richardson RA, Davidson HIM, Hinds A, Cowan S, Rae P, Garden OJ. (1999)  
The influence of the metabolic sequelae of liver cirrhosis on nutritional intake.  
American Journal of Clinical Nutrition 69: 331-337.
- Robertson JD, Reid DD. (1952)  
Standards for the basal metabolism of normal people in Britain.  
Lancet i: 940-943.
- Roche AF, Sienogal RM, Chumlea WC, Webb P. (1981)  
Grading body fatness from limited anthropometric data.  
American Journal of Clinical Nutrition 34: 2831-2838.
- Romijn JA, Endert E, Sauerwein HP. (1991)  
Glucose and fat metabolism during short-term starvation in cirrhosis.  
Gastroenterology 100: 731-739.
- Roth D, Milgrom M, Esquenazi V, Fuller L, Burke G, Miller J. (1989)  
Post-transplant hyperglycaemia.  
Transplantation 47: 278-281.
- Rowland N, Morien A, Li BH. (1996)  
The physiology and brain mechanisms of feeding.  
Nutrition 12: 626-639.
- Rubner M. (1883)  
Über den Einfluss der Körpergröße auf Stoff- und Kraftwechsel.  
Z. Biol. 19: 535-562.
- Saunders JB, Walters JRF, Davies P, Paton A. (1981)  
A 20 year prospective study of cirrhosis.  
British Medical Journal 282: 263.
- Scharrer E, Del Prete E, Rossi R, Langhans W. (1993)  
Metabolic regulation of food intake.  
Proceedings of the Society of Nutrition and Physiology 1: 35-41.
- Schneeweiss B, Graninger W, Ferenci P et al (1990)  
Energy metabolism in patients with acute and chronic liver disease.  
Hepatology 11: 387-393.
- Schoeller DA. (1996)  
Hydrometry.  
In: Human Body Composition.  
Eds. Roche AF, Heymsfield SB, Lohman TG.  
Pub: Human Kinetics, Leeds, UK. pp 25-43.

- Schofield WN. (1985)  
 Predicting basal metabolic rate: new standards and review of previous work.  
 Human Nutrition 39c: 5-41.
- Sclafani A. (1990)  
 Nutritionally based learned flavour preferences in rats.  
 In: Taste, Feeding and Experience.  
 Eds. Capaldi ED, Powley TL.  
 Pub: American Psychological Association, Washington, DC.  
 pp 139-156.
- Scottish Liver Transplant Unit (1998)  
 Annual Report.  
 Royal Infirmary of Edinburgh.
- Segal KR, Edano A, Blando L, Pi-Sunyer FX. (1990)  
 Comparison of thermic effects of constant and relative caloric loads in lean and obese men.  
 American Journal of Clinical Nutrition 51: 14-21.
- Segal KR, Burastero S, Chun A, Coronel P, Pierson RN, Wang J. (1991)  
 Estimation of extracellular and total body water by multiple-frequency bioelectrical impedance analysis.  
 American Journal of Clinical Nutrition 54: 26-29.
- Selberg O, Bottcher J, Tusch G, Pichlmayr R. et al (1997)  
 Identification of high and low risk patients before liver transplantation: a prospective cohort study of nutritional and metabolic parameters in 150 patients.  
 Hepatology 25: 652-657.
- Shanbhogue RLK, Bistrain BR, Jenkins RL, Jones C, Benotti P, Blackburn GL. (1987)  
 Resting energy expenditure in patients with end-stage liver disease and in normal population.  
 Journal of Parenteral and Enteral Nutrition 11: 305-308.
- Sharief NN, MacDonald I. (1982)  
 Differences in dietary-induced thermogenesis with various carbohydrates in normal and overweight men.  
 American Journal of Clinical Nutrition 35: 267-272.
- Shizgal HM. (1983)  
 Body composition.  
 In: Surgical Nutrition  
 Eds: Fischer J.  
 Pub: Little Brown, Boston. pp 3-16.

- Silva G, Gomis R., Bosch J, Casamitjana R, Mastai R, Navasa M, Rivera F. et al. (1988)  
Hyperglucagonism and glucagon resistance in cirrhosis.  
*Journal of Hepatology* 6: 325-331.
- Smith FR, Henkin RJ, Dell RB. (1976)  
Disordered gustatory acuity in liver disease.  
*Gastroenterology* 70: 568-571.
- Smith-Laing G, Sherlock S, Faber OK. (1979)  
Effects of spontaneous portal-systemic shunting on insulin metabolism.  
*Gastroenterology* 76: 685-690.
- Starzl TE, Fung J, Jordan M, Shapiro R, Tzakis A, McCauley J, Johnston J, Iwaki Y, Jain A, Alessiani M, Todo S. (1990)  
Kidney transplantation under FK506.  
*Journal of the American Medical Association* 264: 63-67.
- Stegall MD, Everson G, Schroter G, Bilir B, Karrer F, Kam I. (1995)  
Metabolic complications after liver transplantation.  
*Transplantation* 60: 1057-1060.
- Steiger U, Lippuner E, Jensen X, Montandon A, Jaeger P, Horber F. (1995)  
Body composition and fuel metabolism after kidney grafting.  
*European Journal of Clinical Investigation* 25: 809-816.
- Stock MJ. (1980)  
Effects of fasting and refeeding on the metabolic response to a standard meal in man.  
*European Journal of Applied Physiology* 42: 35-40.
- Tabasco-Minguillan J, Miele L, Carroll P, Gavaler J et al (1993)  
Insulin requirements after liver transplantation and FK-506 immuno-suppression.  
*Transplantation* 56: 862-867.
- Takala J, Keinanen O, Vaisanen P, Aarno K. (1989)  
Measurement of gas exchange in intensive care: laboratory and clinical validation of a new device.  
*Critical Care Medicine* 17: 1041-1047.
- Taylor R, Heine RJ, James OFW, Alberti KGMM. (1985)  
Insulin action in cirrhosis.  
*Hepatology* 5: 64-71.
- Tempel DL, Shor-Posner G, Dwyer D, Leibowitz SF. (1989)  
Nocturnal patterns of macronutrient intake in freely feeding and food deprived rats.  
*American Journal of Physiology* 256: R541-R548.

- Thomas CD, Peters JC, Reed GW, Abumrad NN, Sun M, Hill JO. (1992)  
Nutrient balance and energy expenditure during ad libitum feeding of high-fat and high-carbohydrate diets in humans. *American Journal of Clinical Nutrition* 55: 934-942.
- Thuluvath PJ, Triger DR (1994)  
Evaluation of nutritional status by using anthropometry in adults with alcoholic and non-alcoholic liver disease. *American Journal of Clinical Nutrition* 60: 269-273.
- Todorovic VE, Micklewright A. (1997)  
Assessment 2.  
In: A Pocket Guide to Clinical Nutrition.  
Pubs. British Dietetic Association. pp 2-9.
- Tordoff MG, Friedman MI. (1986)  
Hepatic portal glucose infusions decrease food intake and increase food preference.  
*American Journal of Physiology* 251: R192-R196.
- Tordoff MG, Ulrich PM, Sandler F. (1990)  
Flavour preference and fructose: evidence that the liver detects the unconditioned stimulus for calorie based learning.  
*Appetite* 14: 29-44).
- Tranberg KG. (1979)  
Hepatic uptake of insulin in man.  
*American Journal of Physiology* 237: E509-E518.
- Tremblay A, Levallee N, Almévas N, Allard L, Després J-P, Bouchard C. (1991)  
Nutritional determinants of the increase in energy intake associated with a high-fat diet.  
*American Journal of Clinical Nutrition* 53: 1134-1137.
- Van der Weele DA, Pi-Sunyer FX, Novin D. et al (1980)  
Chronic insulin infusion suppresses food ingestion and body weight gain in rats.  
*Brain Research Bulletin* 5, 7-11.
- Van Thiel DH, Iqbal M, Jain A, Fung J, Todo S, Starzl TE. (1990)  
Gastrointestinal and metabolic problems associated with immunosuppression with either CyA or FK506 in liver transplantation.  
*Transplantation Proceedings* 22: 37-40.
- Verboeket WPHG, Westerterp KR, van Hoek B, Swart GR (1995)  
Energy expenditure and substrate metabolism in patients with cirrhosis of the liver: effects of the pattern of food intake.  
*Gut* 36: 110-116.

- Vohra SB, Keegan MA, Campbell IT, McGuiness K. (1995)  
Practical limitations of the Deltatrac indirect calorimeter.  
Clinical Nutrition 14: 155-161.
- Webber J, MacDonald IA. (1995)  
Energy Metabolism.  
In: Artificial Nutrition Support in Clinical Practice.  
Ed: Payne-James J, Grimble G, Silk D. 1st edition  
Pub: Edward Arnold, London. pp 43-57.
- Weir JBdeV (1949)  
New methods for calculating metabolic rate with special  
reference to protein metabolism.  
Journal of Physiology 109: 1-9.
- Wicks C, Bray GP, Williams R. (1995)  
Nutritional assessment in primary biliary cirrhosis: the effect  
of disease severity.  
Clinical Nutrition 14: 29-34.
- Wilding J. (1997)  
Science, medicine and the future. Obesity treatment.  
British Medical Journal 315: 997-1000.
- Woods RP, Ozaki CF, Katz SM, Monsour HP, Dyer CH,  
Johnston TD. (1994)  
Liver transplantation. The last ten years.  
Surgical Clinical of North America 74: 1133-1154.
- Zhang Y, Proenca R, Maffei M, Barone M, Leopold L, Friedman JM.  
(1994)  
Positional cloning of the mouse obese gene and its human  
homologue.  
Nature 372: 425-432.

## APPENDIX

# Influence of the metabolic sequelae of liver cirrhosis on nutritional intake<sup>1-3</sup>

Rosemary A Richardson, H Isobel Davidson, Alison Hinds, Steven Cowan, Peter Rae, and O James Garden

## ABSTRACT

**Background:** The liver plays a central role in ingestive behavior; alterations in metabolic signaling to the brain stem as a result of chronic liver disease could influence intake.

**Objective:** We examined the influence of metabolic sequelae of liver disease on nutrient intake and nutritional status.

**Design:** Nutritional status and spontaneous dietary intake were examined in 65 cirrhotic patients and 14 control subjects. The response to feeding was investigated in 14 control subjects and a subgroup of 31 cirrhotic patients. Comparisons were made between patients with primary biliary cirrhosis (PBC) and hepatocellular cirrhosis (HC).

**Results:** Patients were nutritionally depleted. The fasting rate of lipid oxidation in the HC group was greater than in the control group ( $P < 0.01$ ). In the fasting state, only HC patients were hyperinsulinemic [ $121.2 \pm 78.5$  compared with  $41.3 \pm 18.6$  pmol/L in control subjects ( $P < 0.001$ ) and  $64.7 \pm 15.8$  pmol/L in PBC patients ( $P < 0.05$ )] and this persisted during the response to feeding. In the fed state, the magnitude of change in carbohydrate oxidation was greatest in the HC group (HC: 34.6%; control: 23.1%; PBC: 25.2%). Carbohydrate and energy intakes of the HC group were lower than in control subjects (carbohydrate:  $193 \pm 38.3$  compared with  $262 \pm 48.1$  g/d,  $P < 0.05$ ; energy:  $6.29 \pm 1.40$  compared with  $9.0 \pm 2.12$  MJ/d,  $P < 0.05$ ).

**Conclusions:** Reductions in carbohydrate intake could be mediated by hyperinsulinemia and compounded by preferential uptake of carbohydrate. This may enhance gastrointestinal satiety signaling and contribute to hypophagia. *Am J Clin Nutr* 1999;69:331-7.

**KEY WORDS** Liver cirrhosis, primary biliary cirrhosis, hepatocellular cirrhosis, substrate oxidation, metabolic regulation, ingestive behavior, carbohydrate intake, humans

## INTRODUCTION

The nutritional and metabolic consequences of cirrhosis have attracted considerable interest over the past decade (1-5); given the central role of the liver in metabolism, it is not surprising that undernutrition is common in chronic liver disease (6-8). In recent years, the focus of research in this area has been on the identification of clinical or biochemical markers associated with nutritional risk (9, 10). These studies have predominantly considered the relation between factors such as pathology, disease

staging, energy status, body-composition status, and substrate oxidation (11, 12); most studies were performed in subjects admitted to the hospital for disease management.

There is emerging interest in the liver as an organ involved in the metabolic control of eating (13-15). Normally, after the delivery of nutrient-rich blood from the portal vein to the hepatocytes, the quantity and quality of substrate oxidation activates hepatic metabolic signaling (via the hepatic afferent nerves) to the brain stem, which influences eating behavior (16). However, with progressive damage, pathophysiologic changes produce alterations in hepatic substrate oxidation, which may cause aberrant metabolic signaling. This phenomenon influences the quantity and quality of nutrients ingested, which consequently affects nutritional status.

In cholestatic cirrhosis [primary biliary cirrhosis (PBC)] or hepatocellular cirrhosis (HC; alcoholic liver disease, hepatitis C, or cryptogenic cirrhosis) the histologic picture is different. PBC is characterized by inflammation of the biliary canaliculi and HC involves primary hepatocellular damage. It was shown previously that the metabolic profile of cirrhosis is influenced by etiology (17, 18). Given that cirrhotic patients represent a heterogeneous population, both the selection and characterization of patients is important in clinical and metabolic studies. In these cases in which nutrient handling and substrate metabolism may influence dietary intake, which in turn would affect nutritional status, there are limited data on the metabolic response to feeding in homogeneous groups of cirrhotic patients.

To examine the possible mechanisms responsible for the undernutrition reported in this patient population, we investigated the influence of liver disease on nutrient intake. We considered nutritional status, metabolic response to a test meal, and spontaneous nutritional intake in stable cirrhotic patients referred for assessment of orthotopic liver transplantation and

<sup>1</sup> From the Department of Dietetics and Nutrition, Queen Margaret College, University Department of Surgery, Royal Infirmary, Edinburgh, and the Departments of Medical Physics and Clinical Chemistry, Western General Hospital, Edinburgh.

<sup>2</sup> Sponsored by a research grant from Nutricia Ltd, Trowbridge, United Kingdom.

<sup>3</sup> Address reprint requests to RA Richardson, University Department of Surgery, Royal Infirmary, Edinburgh EH3 9YW, United Kingdom. E-mail: s.keggie@ed.ac.uk.

Received January 13, 1998.

Accepted for publication July 15, 1998.



made comparisons between PBC and HC patients and with a matched group of healthy control subjects.

## SUBJECTS AND METHODS

Patients admitted to the Scottish Liver Transplant Unit between October 1996 and August 1997 for consideration of orthotopic liver transplantation were eligible for the study. Patients with alcohol-related disease were required to be abstinent for  $\geq 6$  mo. Patients with diabetes, significantly impaired renal or respiratory function, thyroid dysfunction, active inflammatory bowel disease, sepsis, severe ascites, or grade 3–4 encephalopathy were excluded from the study. A group of 14 healthy volunteers was also recruited. The study protocol was reviewed and approved by the local ethical committee. All procedures relating to the study were explained to subjects, who were required to give their written consent.

### Assessment of disease severity and nutritional status

#### *Disease severity*

Disease severity was scored by using Pugh et al's (19) modification of Child's classification. All patients underwent ultrasound scanning as part of the assessment; this confirmed the clinical assessment of the amount of ascites.

#### *Anthropometry*

Subjects' heights were measured without shoes by using a stadiometer (Docherty Medical, London). Subjects were weighed in light clothing to an accuracy of 100 g on mobile sitting scales (Weymed Ltd, London). Body mass index was calculated and patients were asked to recall any weight loss during the preceding year; recent weight loss (RWL) was expressed as a percentage of actual body weight. Triceps skinfold thickness (TSF) and arm muscle circumference (AMC) were measured on 3 occasions by the same trained observer (RAR) to reduce error. Results were averaged and expressed as a percentage of standard values to permit comparisons between men and women (20).

#### *Multifrequency bioelectrical impedance analysis*

This was performed on the day of investigation by using a Xitron 4000B analyzer (Xitron Technologies, San Diego) operated at 200  $\mu$ A at previously defined optimal frequencies of 5 and 200 kHz (21). The alternating current was passed between a set of pregelled current injection electrodes placed on the right hand and foot, proximal to the third metacarpal and metatarsal bones, respectively. Similar detection electrodes were placed on the right wrist between the radius and ulna and on the right ankle between the malleoli. Measurements were taken on the morning of study with the subject in a supine position with his or her legs and leads apart. Total body and extracellular water were calculated from predictive equations derived previously from isotopic studies of surgical patients performed in our unit (22) and body cell mass (BCM) was derived from the equations of Shizgal (23). Bioelectrical impedance analysis in patients with mild and moderate ascites has been validated as a method of assessing the metabolically active component of body composition, the BCM, in a cirrhotic patient population (4).

#### *Indirect calorimetry*

Measurements of oxygen consumption (pragmatic oxygen sensor) and carbon dioxide production (infrared carbon dioxide

sensor) were performed by using a Datex Deltatrac Metabolic Monitor (Engstrom Ltd, Helsinki). Subjects were placed under a ventilated plastic hood and room air was delivered at a constant flow rate (41 L/min). Monthly calibrations to check the flow rate and respiratory quotient were carried out by using an alcohol burning kit (variation between calibrations was  $<2.0\%$ ). Throughout the study, all subjects remained in bed in a quiet area of the ward. After subjects had fasted overnight (10 h), measurements were made over 40 min (10 min of acclimatization and 30 min of calorimetry); in the fed state, metabolic measurements were taken at 4 time points over a period of 2 h and 15 min (3 min of acclimatization and 10 min of calorimetry). Minute-by-minute readings were taken and averaged over each measurement period. Two timed urine collections were made, one over the study period to determine protein oxidation and a 24-h collection to allow calculation of the nonprotein respiratory quotient (NPRQ) from urinary nitrogen analysis (LECO analyzer, Chicago). Measured energy expenditure (MEE) and substrate oxidation were derived from the equations of de Weir (24) and Acheson et al (25), respectively.

#### *Test meal*

After fasting calorimetry was performed, subjects ingested the meal within a 5-min period. Those subjects being studied in the fed state (31 patients, 14 control subjects) were given a palatable and easily prepared dietary challenge (Fortisip and Polycal; Nutricia Ltd, Trowbridge, United Kingdom: 60% carbohydrate, 30% fat, and 10% protein) that provided 15 kJ/kg body wt. This intake ensured that the maximum metabolic effect of the meal would be observed within the study time frame.

#### *Dietary intake*

To assess subjects' usual dietary intake, food diaries (recording intake for 2 weekdays and 1 weekend day) were completed after discharge. Diaries were analyzed with a computerized dietary analysis program (COMP-EAT; Nutrition Systems, London). To minimize error, subjects were fully instructed on how to record intake by the research dietitian (RAR). To ensure that subjects understood how to complete the diet diaries, they were questioned at the end of their interviews. If the diaries were not returned within 1 wk of discharge, subjects were reminded twice to do so by telephone. Diet diaries were analyzed and results averaged to give an estimate of daily macronutrient and energy intakes.

#### *Blood analysis*

Blood samples were taken from an indwelling cannula (Venflon; Ohmeda, Helsingborg, Sweden) in the brachial vein that was kept patent with a minimal infusion of normal saline. Blood samples were taken to measure glucose at baseline and every 20 min for 2 h. Insulin was measured at baseline, 60 min, and 120 min. Plasma glucose was measured by the timed endpoint method (Cobas MIRA Plus; Roche Diagnostics Ltd, Lewes, United Kingdom) and plasma insulin was measured by a 2-site immunoassay (AIA-pack IRI assay, AIA-600 enzyme immunoassay analyzer; Tosoh Corporation, Tokyo).

#### *Statistics*

Data were analyzed by using STATVIEW (version 4.02; Abacus Concepts, Berkeley, CA). Results are expressed as means  $\pm$  SDs. Differences between groups were determined by



TABLE 1

Clinical and anthropometric assessment of cirrhotic patients and control subjects<sup>1</sup>

	Patients			Control subjects (n = 5 M, 9 F)
	All (n = 25 M, 40 F)	PBC (n = 3 M, 31 F)	HC (n = 22 M, 9 F)	
Number with ascites	20	9	11	0
Age (y)	54.6 ± 8.5 <sup>2</sup>	57.7 ± 6.9	51.2 ± 8.9	49.5 ± 11.0
BMI (kg/m <sup>2</sup> )	24.1 ± 4.7	23.5 ± 5.8	24.7 ± 3.5	26.0 ± 3.7
RWL (%)	-7.0 ± 9.7	-7.7 ± 8.4	-6.3 ± 11.3	0
TSF (%)	81 ± 34.1 <sup>3</sup>	82 ± 37.2 <sup>4</sup>	79 ± 31.0 <sup>3</sup>	113 ± 39.0
AMC (%)	94 ± 12.9 <sup>5</sup>	94 ± 15.0 <sup>5</sup>	93 ± 10.5 <sup>5</sup>	105 ± 9.7
BCM (kg)	23.1 ± 5.7	20.1 ± 4.9 <sup>6</sup>	26.1 ± 4.7	24.8 ± 5.2
Child's score	8.5 ± 2.6	7.6 ± 2.8	9.2 ± 2.3	—

<sup>1</sup>PBC, primary biliary cirrhosis; HC, hepatocellular cirrhosis; RWL, recent weight loss, expressed as percentage of actual body weight; TSF, triceps skin-fold thickness, expressed as percentage of standard values (20); AMC, arm muscle circumference, expressed as percentage of standard values; BCM, body cell mass; Child's score as modified by Pugh et al (19).

<sup>2</sup> $\bar{x} \pm SD$ .

<sup>3-5</sup>Significantly different from control subjects: <sup>3</sup> $P < 0.005$ , <sup>4</sup> $P < 0.01$ , <sup>5</sup> $P < 0.05$ .

<sup>6</sup>Significantly different from HC patients,  $P < 0.005$ .

using Student's *t* test for 2 groups and analysis of variance (ANOVA) for 3 groups. The effects of the test meal were analyzed by two-factor repeated-measures ANOVA. Scheffe's test was used to compare means. The linear relation between MEE and BCM was determined by using regression analysis and the method of least squares was used to derive correlation coefficients (*r*). The limit for significance was at the 5% level.

## RESULTS

Sixty-five clinically stable patients with histologic confirmation of the diagnosis of cirrhosis were included in the study of clinical and nutritional status. Clinical and nutritional data are shown in Table 1. There were more women in the PBC group and more men in the HC group. The severity of liver disease was similar in both patient groups. In the patient groups, pharmacologic regimens during the assessment period included diuretic therapy (41 patients), vitamin supplements (13 patients), and lactulose (12 patients).

Energy expenditure was measured by indirect calorimetry and, given that BCM is the oxygen-consuming component of body composition, the relation between MEE and BCM was examined in both patients and control subjects. There was a strong, positive correlation between MEE and BCM in both patients and control subjects. In addition, this relation held in patients with mild and moderate ascites and there were no significant differences in the slopes of the regression lines between any groups (Figure 1). This finding confirms that multifrequency bioelectrical impedance analysis is of value for estimating body composition in populations of cirrhotic patients with mild and moderate ascites.

Endogenous fat reserves and muscle mass, determined by measuring TSF and AMC, respectively, were significantly lower in cirrhotic patients than in control subjects (for all patients combined:  $P < 0.005$  for TSF and  $P < 0.05$  for AMC; for the PBC group:  $P < 0.01$  for TSF and  $P < 0.05$  for AMC; and for the HC group,  $P < 0.005$  for TSF and  $P < 0.05$  for AMC). There was no significant difference in BCM between control subjects and the PBC and HC groups. BCM was significantly lower, however, in the PBC group than in the HC group ( $P < 0.005$ ) and accounted for 31% of body weight, which is within the normal range for

women of this age (26). The mean RWL did not exceed 10% of body weight in any group.

Substrate oxidation rates during fasting and feeding are shown in Table 2. In the PBC group, the NPRQ and macronutrient metabolism were not significantly different from that in control

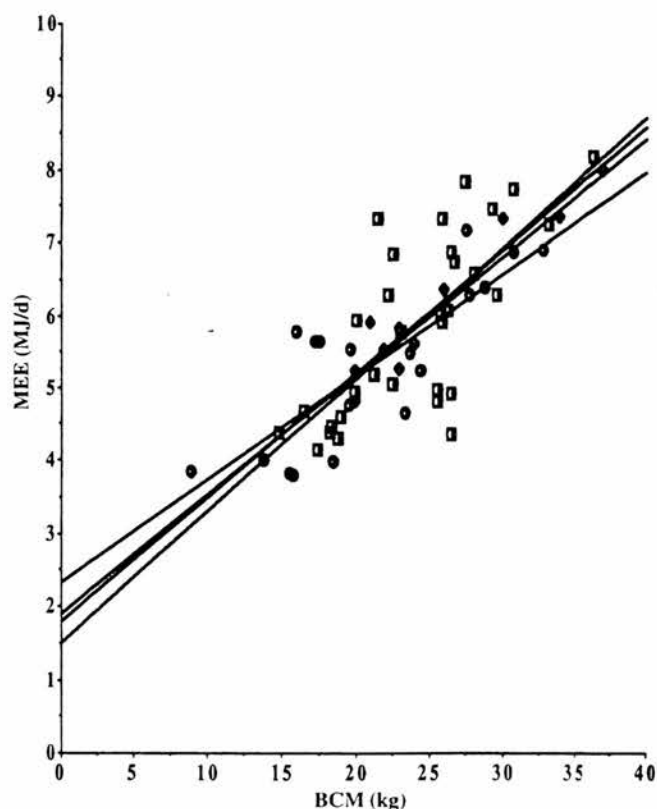


FIGURE 1. Relation between measured energy expenditure (MEE) and body cell mass (BCM) in cirrhotic patients and control subjects. There was a linear relation in all groups and the best-fitting line was determined by linear regression (○, all patients,  $r = 0.765$ ,  $P < 0.0001$ ; Δ, patients with no ascites,  $r = 0.732$ ,  $P < 0.0001$ ; □, patients with ascites,  $r = 0.809$ ,  $P < 0.0001$ ; ◇, control subjects,  $r = 0.952$ ,  $P < 0.0001$ ).

**TABLE 2**  
Substrate oxidation during fasting and feeding in cirrhotic patients and control subjects<sup>1</sup>

	PBC patients (n = 18)		HC patients (n = 13)		Control subjects (n = 14)	
	Fasting	Feeding	Fasting	Feeding	Fasting	Feeding
NPRQ	0.82 ± 0.04	0.89 ± 0.06	0.78 ± 0.03 <sup>2</sup>	0.89 ± 0.08	0.84 ± 0.04	0.93 ± 0.06
Carbohydrate						
(mg/min)	88.6 ± 32.5	156.2 ± 72.6	80.5 ± 34.6	188.0 ± 71.6	119.0 ± 47.1	186.0 ± 59.5
(%)	37.0 ± 12.2	62.2 ± 15.1	29.7 ± 11.1 <sup>2</sup>	64.3 ± 25.6	46.2 ± 13.8	69.3 ± 13.6
Fat						
(mg/min)	46.3 ± 19.5	28.3 ± 17.1	66.7 ± 13.0 <sup>2,3</sup>	34.0 ± 27.2	42.1 ± 8.21	18.4 ± 18.6
(%)	47.5 ± 13.7	27.3 ± 16.0	59.1 ± 12.3 <sup>2</sup>	27.5 ± 22.9	39.3 ± 11.8	14.7 ± 15.5
Protein						
(mg/min)	30.8 ± 14.0	29.0 ± 17.9	29.2 ± 16.4	25.2 ± 19.6	36.9 ± 12.5	45.5 ± 23.0
(%)	14.2 ± 6.5	12.0 ± 7.6	11.3 ± 6.4	9.0 ± 9.3	14.6 ± 3.4	16.4 ± 6.7

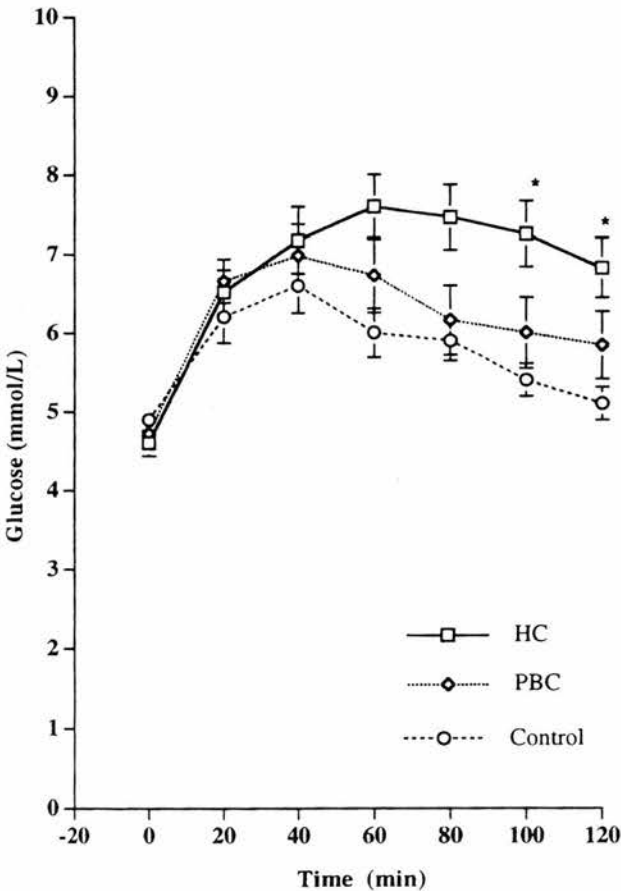
<sup>1</sup>  $\bar{x} \pm SD$ . PBC, primary biliary cirrhosis; HC, hepatocellular cirrhosis; NPRQ, nonprotein respiratory quotient.  
<sup>2</sup> Significantly different from control subjects,  $P < 0.01$ .  
<sup>3</sup> Significantly different from PBC patients,  $P < 0.01$ .

subjects, whereas in the HC group, the fasting NPRQ was significantly lower than in control subjects. The fasting lipid oxidation rate of the HC group was significantly higher than in both control subjects and PBC patients. Additionally, when fasting macronutrient oxidation was calculated as a percentage of energy expenditure, the contribution from fat-derived energy was higher and that from carbohydrate-derived energy was lower in the HC

group than in control subjects. Two hours after ingestion of the test meal, there was a switch to carbohydrate oxidation and at this time no significant differences in NPRQ or substrate utilization were observed. From the fasted to fed state, the magnitude of change in carbohydrate oxidation was 23.1% in the control group, 25.2% in the PBC group, and 34.6% in the HC group.

Glucose and insulin responses to the test meal are illustrated in **Figures 2 and 3**, respectively. The peak glucose response in the control group occurred 40 min after ingestion of the test meal. There was no significant difference in the glucose response curve between the PBC group and the control group. There was, however, a significantly higher response in the HC group than in the control group at 100 ( $7.2 \pm 1.6$  compared with  $5.6 \pm 1.1$  mmol/L) and 120 min ( $6.8 \pm 1.5$  compared with  $5.2 \pm 0.95$  mmol/L). Fasting plasma insulin concentrations were significantly higher in the HC group ( $121.2 \pm 78.5$  pmol/L) than in the control group ( $41.3 \pm 18.6$  pmol/L) and the PBC group ( $64.0 \pm 15.8$  pmol/L). Plasma insulin concentrations rose sharply in the HC group 60 min after ingestion of the meal and were significantly different from concentrations in control subjects ( $635.0 \pm 641.2$  compared with  $203.9 \pm 86.8$  pmol/L). The peak insulin response to the meal in the control group was at 60 min. Insulin concentrations in the HC group remained higher and plateaued over 60–120 min when compared with the average concentration over this time period in control subjects (at 120 min:  $629.5 \pm 636.8$  compared with  $95.0 \pm 57.2$  pmol/L). At 120 min after the test meal, there were also significant differences in insulin response between the HC and PBC groups ( $629.5 \pm 636.8$  compared with  $140.5 \pm 97.8$  pmol/L, respectively).

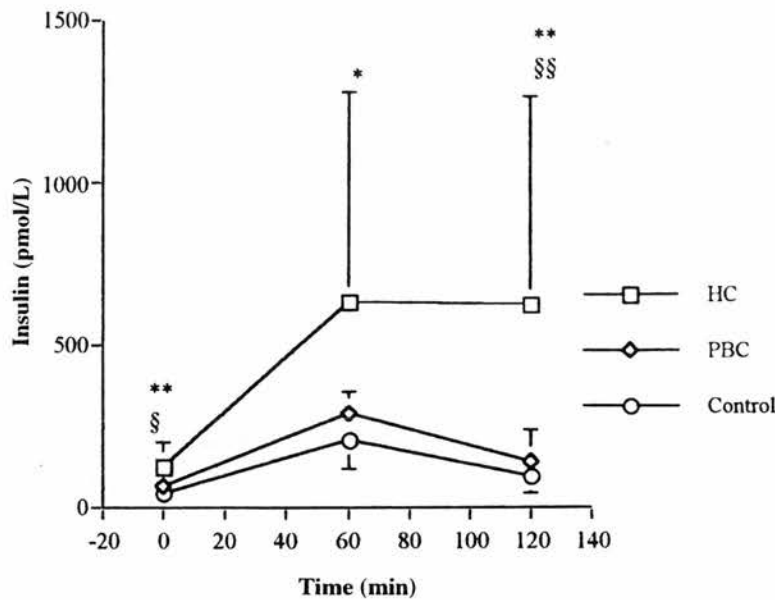
As shown in **Figure 4**, protein intake was significantly lower in the PBC group than in control subjects ( $58.7 \pm 23.1$  compared with  $80.2 \pm 22.3$  g/d), whereas both protein and carbohydrate intakes were significantly lower in the HC group than in control subjects (protein:  $50.4 \pm 11.6$  compared with  $80.2 \pm 22.3$  g/d; carbohydrate:  $193 \pm 38.3$  compared with  $262 \pm 48.1$  g/d). Consequently, total daily energy intake was lower in the HC group than in control subjects ( $6.3 \pm 1.39$  compared with  $9.0 \pm 2.12$  MJ/d; **Figure 5**).



**FIGURE 2.** Mean ( $\pm SD$ ) glucose response to the test meal in cirrhotic patients and control subjects. HC, hepatocellular cirrhosis; PBC, primary biliary cirrhosis. \*Significantly different from control subjects,  $P < 0.05$ .

**DISCUSSION**

Undernutrition in cirrhotic patients and its relation with morbidity and mortality is well documented (7–9). Studies that iden-



**FIGURE 3.** Mean ( $\pm$ SD) insulin response to the test meal in cirrhotic patients and control subjects. HC, hepatocellular cirrhosis; PBC, primary biliary cirrhosis. \*\*Significantly different from control subjects: \* $P < 0.05$ , \*\* $P < 0.001$ . §§Significantly different from PBC patients: § $P < 0.05$ , §§ $P < 0.01$ .

tify the prevalence and degree of undernutrition in chronic liver disease are important in that they permit the targeting of nutritional support to undernourished patients. However, as a result of the metabolic sequelae of cirrhosis, traditional high-energy, high-carbohydrate regimens may not be tolerated by some patients.

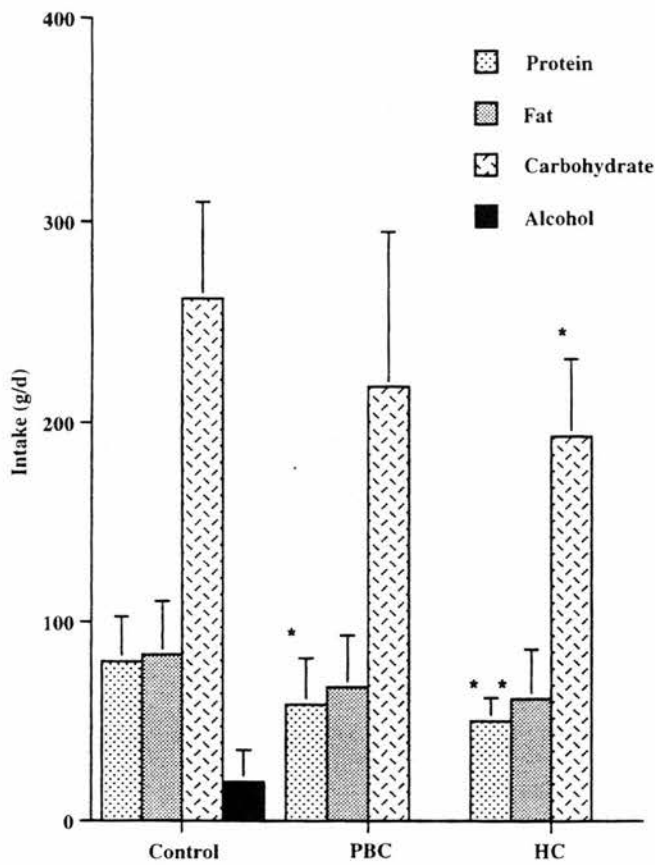
The present study examined the effect of histologically proven cirrhosis on nutritional intake in stable, chronic cirrhosis patients being assessed for liver transplantation. Interpretation of data relating to BMI and RWL can be influenced by the presence of ascites, but in this study there were no significant differences in TSF, AMC, or BCM between patients with and without ascites. The arm was used to estimate fat and muscle reserves because measurements at this site are less affected by fluid shifts (9). In the present study, no patient's ascites was severe enough to warrant drainage, which supports our finding that no patient was grossly ascitic. This study focused predominantly on the metabolic sequelae of cirrhosis and their influences on intake; given that there were no significant differences in those nutritional indexes unaffected by ascites (ie, arm anthropometry) or in the relation between MEE and BCM, patients were stratified by primary diagnosis.

The underlying cause of cirrhosis may influence metabolism. Alcoholic cirrhosis, cryptogenic cirrhosis, and hepatitis C are characterized by hepatocellular damage, whereas PBC is a disease that predominantly affects women in their middle years and is cholestatic in nature. All patients in the study were shown to be depleted in fat and muscle when compared with the normally nourished control group. BCM was lower in patients with PBC than in those with HC but was not significantly different from that in control subjects. This finding may be accounted for by the predominance of women in the PBC group, who normally have a higher ratio of fat to lean tissue than do men. It has been suggested that cirrhotic patients with a BCM  $< 30\%$  of body weight are undernourished (27), which is within the normal range for women in their 50s (26). In our study population, BCM as a percentage of body weight accounted for 31% of PBC, reflecting the sex bias.

The lower fasting NPRQ and higher lipid oxidation rate in the HC group suggests early depletion of hepatic glycogen stores with a concomitant switch to lipolysis and indicates an extended period of fasting. Interestingly, in this study, the HC group had the smallest endogenous fat reserves but the highest rate of fasting lipid oxidation. Conversely, the fasting substrate profile in the PBC group was similar to that in control subjects, suggesting that hepatic glycogen reserves are better preserved in PBC. This increased reliance on endogenous lipids to meet energy requirements in the HC group may be an adaptive process, and one that serves to preserve lean body mass during periods of nutrient deprivation.

In the postingestive period carbohydrate oxidation predominated at rates that were not significantly different between patients and control subjects. Nonetheless, the magnitude of change was greatest in the HC group, suggesting that in this group carbohydrate was preferentially oxidized after ingestion of a mixed meal. This finding supports the work of Levine et al (28), who, when supplying a test meal 3 times as large as the one used in the current study (59 kJ/kg), observed avid oxidation of carbohydrate by patients with alcohol cirrhosis 2 h after ingestion of the meal.

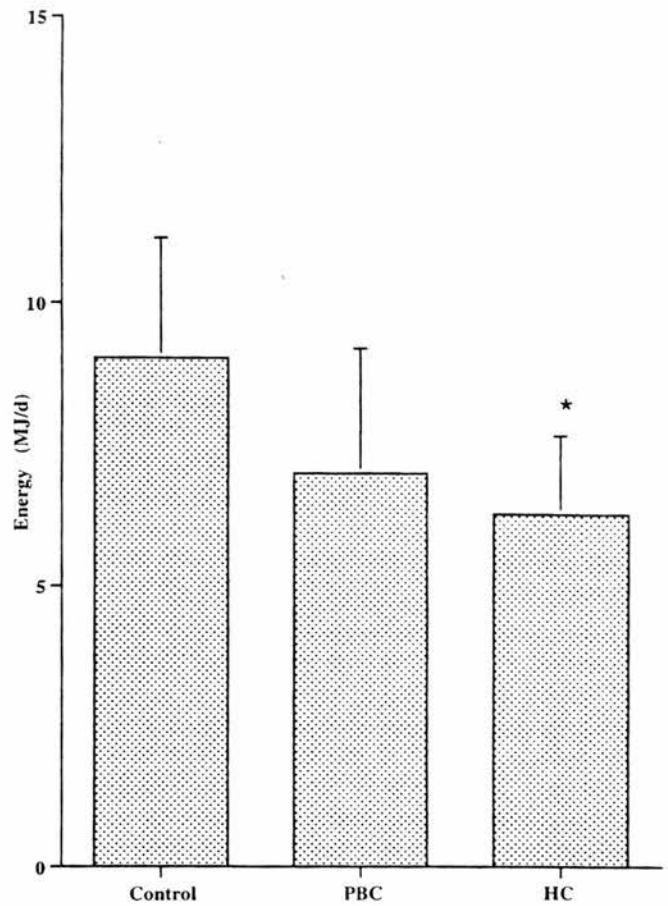
In the fasted state, only the HC group had elevated plasma insulin concentrations. After consumption of the test meal, the PBC group exhibited a normal insulin response that contrasted markedly with the hyperinsulinemia seen in the HC group. The pathogenesis of insulin resistance in cirrhosis is still unknown; several researchers have failed to attribute cirrhotic hyperinsulinemia to portal systemic shunting but rather attributed it to deficits in binding and postbinding insulin target organ cells (17, 29, 30). Earlier work linked hyperinsulinemia with deficient hepatic insulin extraction or the shunting of portal venous blood to the systemic circulation (31). There is, however, no consensus as to the exact mechanisms involved in the hyperinsulinemia of cirrhosis but portosystemic shunting seems unlikely because hyperinsulinemia is not found in patients with portal vein thrombosis and normal liver function (32). In the current study, there



**FIGURE 4.** Mean ( $\pm$ SD) daily macronutrient intake in cirrhotic patients and control subjects. PBC, primary biliary cirrhosis; HC, hepatocellular cirrhosis. \*Significantly different from control subjects:  $P < 0.05$ , \*\* $P < 0.01$ .

were no significant differences in insulin concentrations in patients with ( $n = 10$ ) and without ( $n = 21$ ) evidence of portosystemic shunting.

Blood glucose concentrations were significantly elevated in the HC group toward the end of the postprandial period, whereas the glucose response curve was normal in the PBC group. The different metabolic response to feeding in HC and PBC is an important finding that has not been widely reported. Taylor et al (17) noted impaired glucose tolerance and insulin resistance in cirrhotic patients but, as in the present study, showed that PBC patients were less insulin resistant than patients with HC. These findings support the notion that hepatocyte function is better preserved in PBC. Insulin is known to inhibit dietary intake by stimulating the satiating effects of cholecystokinin and through its direct action in the brain (33). When we examined the ad libitum dietary intake of our study population, all patients—irrespective of the primary etiology of their disease—had significantly lower protein intakes than did control subjects. The reason for this is unclear but could have been the inappropriate prescribing of low-protein dietary regimens by clinicians (27, 34). Interestingly, a lower carbohydrate intake was noted only in the HC group; it can be hypothesized that the defects in carbohydrate metabolism found in this study could bring about a spontaneous reduction in the consumption of this macronutrient. Furthermore, this reduction in carbohydrate intake could have been caused by the presence of hyperinsulinemia and compounded by




**FIGURE 5.** Mean ( $\pm$ SD) daily energy intake in cirrhotic patients and control subjects. PBC, primary biliary cirrhosis; HC, hepatocellular cirrhosis. \*Significantly different from control subjects,  $P < 0.05$ .

the preferential uptake of carbohydrate. This may enhance signaling by the gastrointestinal glucosensors and in turn contribute to hypophagia. Furthermore, the energy intake of the HC group was only about two-thirds that of the control group and this level of intake is consistent with the energy consumption of hospitalized cirrhotic patients (35). If continued over a period of months or years, this reduced intake would contribute significantly to a deterioration in nutritional status. Note that with regard to diet diaries, patients referred to a transplant unit are highly compliant. In addition, through meticulous attention to patient instruction, every effort was made to minimize sources of error (ie, transcription of diaries by RAR).

There is ample evidence from animal studies to support the central role of the liver in controlling ingestive behavior (36). Hepatic metabolic sensors continuously monitor substrate oxidation and relay this information to the brain stem via the hepatic afferent nerves. In this study, we began to study for the first time the effects of the metabolic aberrations of chronic liver disease on eating behavior. The next logical step would be to conduct controlled studies of ingestive behavior to identify clinically acceptable and effective dietary regimens. We conclude that it may be physiologically impossible for patients with HC to follow dietary advice that advocates increasing carbohydrate intake because of the initiation of an amplified satiety cascade mediated by elevated insulin concentrations. Postprandial rises in insulin



are associated with a reduction of hunger and increasing satiety (37). In the HC population, the avid switch to carbohydrate oxidation with feeding, which was maintained in the short-term postprandial period, suggests intracellular uptake of glucose and hence functioning insulin. Early and enhanced postprandial rises in insulin may therefore reduce energy intake through normal satiety mechanisms, which are enhanced in these patients.

Many researchers have considered the relation between nutritional status and liver disease (4, 7, 10). However, as we begin to move into evidence-based nutritional practice, we must develop acceptable and achievable nutritional strategies for cirrhotic patients. Liver transplantation and the hospitalization of cirrhotic patients is expensive clinically, socially, and psychologically. Because research has confirmed that nutritional status is an outcome factor (38), it is of paramount importance to understand the disease-specific nutritional requirements of this patient population. This will only be achieved when the interaction between the metabolic sequelae of primary liver pathology and their influence on ingestive behavior is understood. 

## REFERENCES

- Jhangiani SS, Argarwal N, Holmes R, Cayten CG, Pitchumoni CS. Energy expenditure in chronic alcoholics with and without liver disease. *Am J Clin Nutr* 1986;44:323-9.
- John WJ, Phillips R, Ott L, Adams LJ, McClain CJ. Resting energy expenditure in patients with alcoholic hepatitis. *JPEN J Parenter Enteral Nutr* 1989;13:124-7.
- Green HJ, Bramley PN, Losowsky MS. Are patients with primary biliary cirrhosis hypermetabolic? A comparison between patients before and after liver transplantation and controls. *Hepatology* 1991;14:464-72.
- Muller MJ, Lautz HU, Plogmann B, et al. Energy expenditure and substrate oxidation in patients with cirrhosis: the impact of cause, clinical staging and nutritional state. *Hepatology* 1992;15:782-93.
- Campillo B, Phuong B, Pornin B, Devanlay M. Influence of liver failure, ascites and energy expenditure on the response to oral nutrition in alcoholic liver cirrhosis. *Nutrition* 1997;13:613-21.
- Crawford DHG, Cuneo RC, Shepherd RW. Pathogenesis and assessment of malnutrition in liver disease. *J Gastroenterol Hepatol* 1993;8:89-94.
- Mendenhall CL, Moritz TE, Roselle GA, et al. Protein energy malnutrition in severe alcoholic hepatitis: diagnosis and response to treatment. *JPEN J Parenter Enteral Nutr* 1995;19:258-65.
- Caregaro L, Alberino F, Amodio P, et al. Malnutrition in alcoholic and virus-related cirrhosis. *Am J Clin Nutr* 1996;63:602-9.
- Wicks C, Bray GP, Williams R. Nutritional assessment in primary biliary cirrhosis: the effect of disease severity. *Clin Nutr* 1995;14:29-34.
- Merli M, Riggio O, Dally L, Policentrica Italiana Nutrizione Cirrosi. Does malnutrition affect survival in cirrhosis? *Hepatology* 1996;23:1041-6.
- Muller MJ, Boker KHW, Selberg O. Are patients with liver cirrhosis hypermetabolic? *Clin Nutr* 1994;13:131-44.
- Thuluvath PJ, Triger DR. Evaluation of nutritional status by using anthropometry in adults with alcoholic and nonalcoholic liver disease. *Am J Clin Nutr* 1994;60:269-73.
- Friedman MI, Sawchenko PE. Evidence for hepatic involvement in control of ad libitum food intake in rats. *Am J Physiol* 1984;247:R106-13.
- Langhans W. Hepatic and intestinal handling of metabolites during feeding in rats. *Physiol Behav* 1991;49:1203-9.
- Scharrer E, Del Prete E, Rossi R, Langhans W. Metabolic regulation of food intake. *Proc Soc Nutr Physiol* 1993;1:35-41.
- Nijima A. Afferent impulse discharges from glucoreceptors in the liver of the guinea pig. *Ann N Y Acad Sci* 1982;157:690-700.
- Taylor R, Heine RJ, James OFW, Alberti KGM. Insulin action in cirrhosis. *Hepatology* 1985;5:64-71.
- Orrego H, Blake JE, Blendis LM, Compton KV, Israel Y. Long-term treatment of alcoholic liver disease with propylthiouracil. *N Engl J Med* 1987;317:1421-6.
- Pugh RMH, Murray-Iyon IM, Dawson JL, Pietroni MC, Williams R. Transection of the oesophagus for bleeding oesophageal varices. *Br J Surg* 1973;60:646-9.
- Jeliffe DB. The assessment of the nutritional status of the community. *World Health Organ Monogr Ser* 1996;53.
- Hannan WJ, Cowan SJ, Fearon KCH, Plester CE, Falconer JS, Richardson RA. Evaluation of multi-frequency bio-impedance analysis for the assessment of extracellular and total body water in surgical patients. *Clin Sci* 1994;86:479-85.
- Fearon KCH, Richardson RA, Hannan WJ, et al. Bioelectrical impedance analysis in the measurement of the body composition of surgical patients. *Br J Surg* 1992;79:421-3.
- Shizgal HM. Body composition. In: Fischer J, ed. *Surgical nutrition*. Boston: Little Brown, 1983:3-16.
- de Weir JB V. New methods for calculating metabolic rate with special reference to protein metabolism. *J Physiol* 1949;109:1-9.
- Acheson KJ, Schutz Y, Bessard T, Ravussin E, Jequier E. Nutritional influences on lipogenesis and thermogenesis after a carbohydrate meal. *Am J Physiol* 1984;246:E62-70.
- Cohn SH, Vaswani AN, Yasumura S, Yuen K, Ellis KJ. Improved models for determination of body fat by in vivo neutron activation. *Am J Clin Nutr* 1984;40:255-9.
- Lautz HU, Selberg O, Korberg J, et al. Protein-calorie malnutrition in liver cirrhosis. *Clin Invest* 1992;70:478-82.
- Levine JA, Stanger LC, Morgan M. Resting and post-prandial energy expenditure and fuel utilization in patients with compensated cirrhosis and control subjects. *Clin Nutr* 1989;11:54 (abstr).
- Nolte W, Hartmann H, Ramadori G. Glucose metabolism and liver cirrhosis. *Clin Endocrinol* 1995;103:63-74.
- Baruch Y, Amit T, Hertz P, Enat R, Youdin MBH, Hochberg Z. Decreased serum growth hormone binding protein in patients with liver cirrhosis. *J Clin Endocrinol Metab* 1991;73:777-80.
- Bosch J, Gomis R, Kravetz D, et al. Role of spontaneous portal-systemic shunting in hyperinsulinism of cirrhosis. *Am J Physiol* 1984;247:G206-12.
- Johnston DG, Alberti KGMM, Wright R, Smith-Laing G. C-peptide and insulin in liver disease. *Diabetes* 1978;27:201-6.
- Langhans W. Role of the liver in the metabolic control of eating: what we know—and what we do not know. *Neurosci Biobehav Rev* 1996;20:145-53.
- Soulsby CT, Morgan MY. Is the dietary treatment of hepatic encephalopathy in UK hospitals appropriate? A survey of current practice. *Clin Nutr* 1997;16(suppl):15 (abstr).
- Nielsen K, Kandrup J, Martinsen L, Stilling B, Wikman B. Nutritional assessment and adequacy of dietary intake in hospitalized patients with alcoholic liver cirrhosis. *Br J Nutr* 1993;69:665-79.
- Friedman MI, Tordoff MG, Ramirez I. Integrated metabolic control of food intake. *Brain Res Bull* 1986;17:855-9.
- Raben A, Holst JJ, Christensen NJ, Astrup A. Determinants of post-prandial appetite sensations: macronutrient intake and glucose metabolism. *Int J Obes* 1996;20:161-9.
- Selberg O, Bottcher J, Tusch G, et al. Identification of high and low risk patients before liver transplantation: a prospective cohort study of nutritional and metabolic parameters in 150 patients. *Hepatology* 1997;25:652-7.

# Macronutrient Preference, Dietary Intake, and Substrate Oxidation Among Stable Cirrhotic Patients

H. ISOBEL M. DAVIDSON, ROSEMARY RICHARDSON, DONALD SUTHERLAND, AND O. JAMES GARDEN

Anorexia in liver disease is common; however, its association with aberrant metabolism and the type of cirrhosis has not been considered. Dietary intake, nutritional status, fasting substrate oxidation, and macronutrient preference were examined in controls ( $n = 18$ ) and 65 patients with hepatocellular ( $n = 31$ ) or biliary cirrhosis ( $n = 34$ ). Energy intakes were lowest in hepatocellular patients (controls:  $9.0 \pm 0.48$  megajoules/day compared with biliary:  $7.0 \pm 0.40$  MJ/day,  $P < .05$ ; controls compared with hepatocellular  $6.5 \pm 0.39$  megajoules/day,  $P < .01$ ). Triceps skinfold was lower only in hepatocellular patients (controls:  $109 \pm 9.2\%$  compared with hepatocellular  $79 \pm 5.6\%$ ,  $P < .05$ ). The fasting rate of lipid oxidation was elevated in hepatocellular patients when compared with controls and biliary patients (controls:  $40.9 \pm 15.1$  mg/min compared with hepatocellular  $62.8 \pm 16.8$  mg/min,  $P < .001$ , and biliary:  $45.5 \pm 17.0$  mg/min compared with hepatocellular,  $P < .001$ ). Control subjects exhibited a greater preference for the high fat, moderate carbohydrate food (controls: median 7.0 IQR 2.0 compared with biliary: median 5.0 interquartile range [IQR] 4.7,  $P < .01$ ) (controls compared with hepatocellular: median 6.0 IQR 4.0,  $P < .01$ ). Cirrhotic patients' spontaneous dietary intake is lower than that of controls and recommended intakes. Although macronutrient preference ratings were different within cirrhotic patient groups it remains unclear whether associated nutrient deficits are metabolically driven and dictated by primary cause. (HEPATOLOGY 1999;29:1380-1386.)

The metabolic features of chronic liver disease and the prevalence of undernutrition have been widely reported.<sup>1-4</sup> However, there remains some debate as to the impact of disease severity, primary cause, and metabolic indices on nutritional status.<sup>5-8</sup> It is clear that the presence of undernutrition will impact negatively on clinical outcome.<sup>9-10</sup> Given this significance of nutritional status, it is surprising that few studies have examined the relationship of chronic liver

disease with anorexia, a factor contributing to poor nutritional status.<sup>11</sup> Poor dietary intake in patients with chronic liver disease has been identified as a major concern and one that is difficult to resolve.<sup>12-13</sup> This may, in part, be a result of the complex interaction between aberrant metabolism occurring in liver disease and ingestive behavior.

It is evident that the liver plays a central role in ingestive behavior<sup>14</sup> acting as a sensor for the metabolic control of eating.<sup>15</sup> Under normal conditions, hepatic oxidation of lipid and glucose results in changes in hepatocyte membrane potential and modulation of spike frequency in afferent pathways, resulting in satiety.<sup>16</sup> Ultimately, it is the convergence in the central nervous system of these neural signals, as well as other humoral and cognitive influences, that determines the size and duration of an eating episode. Impairment of hepatic nerve function and/or alterations in the fuel mix being oxidized may be present in liver disease and could contribute to alterations in ingestive behavior. It has also been suggested that impaired functional capacity of the liver may lead to reduced clearance of humoral mediators of satiety such as cholecystokinin (CCK).<sup>17</sup> This may also contribute to a reduced satiety threshold and bring about early meal termination. In the context of patients with chronic liver disease, the mechanisms involved in the metabolic control of eating may be altered and could contribute to anorexia. Changes in ingestive behavior could also be mediated through hepatic cytokine production.<sup>18</sup> For example, IL-1 $\beta$  has been shown to alter activity in the hepatic vagus,<sup>19</sup> which would cause changes in central processing and be interpreted by the brain as an increase in nutrient availability.

It is apparent that bizarre food preferences exist in the presence of chronic liver disease, with the development of cravings for specific foods such as grapefruit having been documented.<sup>20</sup> In a similar study by Madden et al.<sup>21</sup> no preference for specific foods was evident; however, they highlighted a significant increase in the use of vinegar in their cirrhotic patients. The development of these preferences cannot consistently be attributed to any single biochemical parameter and as such has been related with the disease process *per se*. The contribution that these changes in food choice make to total energy intake is difficult to quantify. However, the development of preferences, not for specific food types but for the specific macronutrient composition of that food, may contribute to low energy intake. If this is the case then a reduction in food quality may result in an energy deficit and contribute to the undernutrition found in this population. The palatability of fat and carbohydrate mixtures as real food is more appropriate for studies on macronutrient preference,<sup>22</sup> rather than methodologies that examine detection and recognition taste thresholds,<sup>21,23</sup> or food frequency

Abbreviations: CCK, cholecystokinin; HC, hepatocellular cirrhosis; BC, biliary cirrhosis; R.R., Rosemary Richardson; BMI, body mass index; IQR, inter-quartile range; ESPEN, European Society of Parenteral and Enteral Nutrition; CHO, carbohydrate.

From the Department of Dietetics and Nutrition, Queen Margaret College, University Department of Surgery, Scottish Liver Transplant Unit, Edinburgh, Royal Infirmary, Edinburgh, UK.

Received October 1, 1998; accepted February 18, 1999.

This study was generously supported by a research grant from Nutricia Ltd, UK.

Address reprint requests to: Rosemary Richardson, University Department of Surgery, Edinburgh Royal Infirmary, Lauriston Place, Edinburgh, EH 3 9 YW, UK. E-mail: JDSRIE@ed.ac.uk; fax: 44-131-228-2661.

Copyright © 1999 by the American Association for the Study of Liver Diseases.

0270-9139/99/2905-0006\$3.00/0

questionnaires but has not been examined in this population to date.

Preference for a specific macronutrient composition of a food has not previously been investigated in chronic liver disease. This may be an important determinant of quantitative food intake in chronic liver disease where, as a result of aberrant metabolism, central integration of metabolic information is disrupted. This study examined food preference, macronutrient intake, and fasting substrate oxidation in a group of stable cirrhotic patients.

PATIENTS AND METHODS

Patients admitted for consideration of orthotopic liver transplantation to the Scottish Liver Transplant Unit between October 1996 and December 1997 were eligible for the study. Patients with alcohol-related disease were required to be abstinent for a minimum of 6 months. Patients with diabetes, significantly impaired renal or respiratory function, thyroid dysfunction, active inflammatory bowel disease, sepsis, severe ascites, and grade 3 to 4 encephalopathy were excluded from the study. A group of 18 healthy volunteers were recruited from hospital staff, none of whom were smokers or on any medication or dietary restriction. Disease severity was based on Pugh's modified Child's classification<sup>24</sup> and use the criteria: plasma bilirubin concentration, prothrombin time, presence of ascites, and degree of encephalopathy. Patients were divided into those with predominantly cholestatic (BC) and hepatocellular (HC) liver cirrhosis to enable assessment of the relationship between the type of cirrhosis, nutrient preference, and nutritional status. The study protocol was reviewed and approved by the local ethics committee. All procedures relating to the study were explained to subjects who were required to give written consent.

Energy and Macronutrient Intake

To estimate macronutrient intake subjects were asked to complete a 3-day dietary diary (2 weekdays and 1 day at the weekend). In the present study it was neither practical or logistically possible to determine variance in diet diaries against weighed intakes. However, in a population of a similar size this methodology has previously shown good agreement with weighed intakes.<sup>25</sup> Detailed verbal and written instructions on the completion of diet diaries was issued. A telephone reminder was made if the diary was not returned within 10 days. Transcription of diet diaries was undertaken by the research dietitian (R.R.) and analyzed using a dietary analysis computer program (Comp-eat, Nutrition Systems, London, UK).

Nutritional Status

All subjects were weighed and measured for height to enable calculation of body mass index (weight [kg]/height [m<sup>2</sup>]; BMI). Patients were asked to recall any weight loss during the preceding year and this was expressed as a percentage of actual body weight (pre-illness weight loss). An index of endogenous fat and muscle reserves was estimated from triceps skinfold thickness and arm muscle circumference measurements and these were compared with standard values.<sup>26</sup> Arm anthropometry results were expressed as a percentage of standard to normalize for gender differences in body composition. All anthropometric measurements were performed on three occasions by the same skilled observer (R.R.) and the average taken as the absolute value. Values for a single parameter less than 80% were used to classify undernutrition.<sup>27</sup>

Substrate Oxidation

In all subjects substrate oxidation rates were calculated from gaseous exchange measurements using indirect calorimetry (Datex, Engstrom, Kent) and nitrogen excretion.<sup>28</sup> To ensure accurate gas exchange measurements calibration of gas sensors using Carbogen (5% CO<sub>2</sub>, 95% O<sub>2</sub>; Datex, Helsinki, Finland) was performed at the beginning of each measurement period. The flow rate and respira-

tory quotient of the calorimeter was checked on a monthly basis using the manufacturer's alcohol burning kit (Datex, Helsinki, Finland) and throughout the study the CV was less than 3%. Following an overnight fast, subjects were placed under a perspex hood and measurements of oxygen consumption and carbon dioxide production were taken every minute for a total of 40 minutes (10 minutes' acclimatization and 30 minutes' calorimetry). All subjects remained in bed in a quiet area of the ward throughout the study period. Urinary nitrogen was measured from a 24-hour urine collection (LECO analyzer, Chicago, IL).

Macronutrient Preference

Four commercially available ice creams (a real food combining fat and carbohydrate) were used as the test stimuli to determine macronutrient preference, using methodology adapted from Drewnowski and Greenwood.<sup>29</sup> Each ice cream varied in macronutrient content but all were vanilla in flavor. The composition of the ice creams used did not change over the study period. On the morning of study approximately 15 mL of ice cream was presented in a plastic beaker which was coded (Table 1) and subjects consumed approximately a third of each sample. Subjects randomly selected samples for tasting and eye shades were worn during the tasting procedure to mask any visual differences. Hedonic responses (degree of liking) were recorded for each coded sample on a nine-point hedonic scale<sup>30</sup> anchored by the responses "dislike extremely" and "like extremely." Therefore the higher the score awarded to the test stimuli the greater the degree of liking for the food. Subjects tasted and swallowed samples and were asked subsequently to elicit pleasantness ratings. Retasting was permitted. Subjects were instructed on palate cleansing that involved rinsing the mouth with bottled water (Evian, France) and this was performed before each tasting session and following ingestion of each sample. A resting period of two minutes was enforced between tasting of each test stimulus. At least 3 hours after study macronutrient preference was re-examined in all subjects and no differences between the first and second tasting were observed.

Statistical Analysis

Data were analyzed using Statview 4.02 (Albus Concepts, CA). Substrate oxidation rates, nutritional status, and dietary analysis are expressed as mean (± SEM). Differences between groups were evaluated using Student's *t*-test and one way analysis of variance (ANOVA). Scheffe's test was used in comparing means. Food preference results were expressed as median and interquartile range (IQR) and values out with the 25<sup>th</sup> and 75<sup>th</sup> percentiles were represented in figures individually. Differences between groups were determined using Kruskal-Wallis one-way analysis of variance and Mann-Whitney *U* test. Differences within groups were determined using Friedman's two-way analysis and Wilcoxon signed rank test. Linear regression analysis was performed using the method of least

TABLE 1. Macronutrient Values for Ice Creams (/100 mL)

Sample Code	Wt/100 mL	Protein (g)	Fat (g)	CHO (g)	Energy (KJ), (kcal)	Type
A	166	6.5	19.0	29.7	1339 (321)	High fat, high CHO
B	114	4.5	18.4	20.0	1078 (258)	High fat, mod CHO
C	181	3.8	0.9	27.8	564 (135)*	Low fat, high CHO
D	217	2.8	4.8	20.8	589 (141)	Low fat, mod CHO

NOTE. A, Mackie's Traditional; Westertown, Aberdeen, UK; B, Haagen Dazs, Middlesex, UK; C, Walls Too Good To Be True, Birds Eye Walls Ltd., Surrey, UK; D, Heinz Weight Watchers, HJ Heinz Co. Ltd., Middlesex, UK.



squares and correlation coefficients (*r*) derived (Spearman Rank). The limit for significance was at the 5% level.

## RESULTS

Sixty-five patients with histological confirmation of cirrhosis were included in the study. Demographic, clinical, and nutritional assessment details are shown in Tables 2 and 3. In the HC group, 24 patients had alcohol-related disease, 5 had cryptogenic cirrhosis, and 2 hepatitis C. Three patients (5%) admitted to being smokers and all control subjects were nonsmokers. Thirty patients were classified as normally nourished (16 BC, 14 HC) and 35 undernourished (18 BC, 17 HC). Comparative analysis of nutritional assessment data showed that undernourished patients had significantly lower triceps skinfold measurements when compared with their normally nourished counterparts (normally nourished:  $105.3 \pm 5.9\%$  versus undernourished:  $57.6 \pm 2.8\%$ ,  $P < .0001$ ). No difference in arm muscle circumference was seen. No patient had severe ascites at the time of study and although there were differences in nutritional indices compared with the control group these did not reach statistical significance. Patients with BC were significantly lighter than control subjects but there were no differences between groups when BMI was calculated. Triceps skinfold thickness was significantly lower in the HC group compared with controls. No significant differences in any of the other nutritional variables were observed.

Completed diet diaries were returned from 52 patients (80%). The subject's dietary analysis was compared with the recommended intake for a 67-kg patient with cirrhosis (average weight of patient in current study) using ESPEN (European Society of Parenteral and Enteral Nutrition) nutritional guidelines<sup>31</sup> (Table 4). When compared with controls, cirrhotic patients had lower protein ( $P < .001$ ), fat ( $P < .01$ ), carbohydrate ( $P < .01$ ), and energy ( $P < .001$ ) intakes. In addition when patients were stratified by primary etiology, energy and protein intakes were significantly lower in both BC and HC patients when compared with controls (energy: BC,  $P < .05$ ; HC,  $P < .01$ ; protein: BC,  $P < .05$ ; HC,  $P < .001$ ). The HC group exhibited significantly lower fat ( $P < .05$ ) and CHO intakes ( $P < .05$ ) when compared with control subjects. The energy and protein intakes of cirrhotic

patients were suboptimal when compared with ESPEN guidelines. No differences in dietary intake were seen between cirrhotic patients classified as normally nourished or undernourished.

Fasting protein and glucose oxidation rates were significantly lower in the cirrhotic group when compared with controls (protein: controls,  $38.3 \pm 3.4$  mg/min versus cirrhotic patients,  $27.8 \pm 3.6$  mg/min,  $P < .01$ ; glucose: controls,  $120.9 \pm 10.7$  mg/min versus cirrhotic patients,  $87.1 \pm 3.9$  mg/min,  $P < .001$ ) and the lipid oxidation rate was significantly higher in cirrhotic patients (lipid: controls,  $40.9 \pm 3.6$  mg/min versus cirrhotic patients,  $53.4 \pm 2.6$  mg/min,  $P < .05$ ). No effect of disease severity on substrate oxidation rate was observed when patients were stratified using Pugh's modified Child's score (Fig. 1). In addition no differences in fasting fuel oxidation rates were observed when patients were subdivided by nutritional status (Fig. 1). When cirrhotic patients were grouped by primary diagnosis, the BC group exhibited a depressed rate of glucose oxidation when compared with control subjects (controls,  $120.9 \pm 10.7$  mg/min versus BC,  $87.8 \pm 5.6$  mg/min,  $P < .01$ ). There was no difference in the rate of lipid oxidation between controls and BC patients. The HC patients had decreased rates of protein and glucose oxidation (protein: controls,  $38.3 \pm 3.4$  mg/min vs. HC,  $26.3 \pm 2.6$  mg/min,  $P < .05$ ; glucose: controls,  $120.9 \pm 10.7$  vs. HC,  $86.4 \pm 5.6$  mg/min,  $P < .01$ ). Additionally, the fasting rate of lipid oxidation rate was elevated in the HC group compared with control subjects or BC patients (controls,  $40.9 \pm 2.9$  mg/min vs. HC,  $62.8 \pm 2.5$ ,  $P < .001$ ; HC vs. BC,  $45.4 \pm 2.9$  mg/min,  $P < .001$ ). Given these differences in substrate oxidation rates by primary diagnosis, analysis of macronutrient preference are presented using this stratification.

### Macronutrient Preference

**Analysis Between Groups.** Studies showed that when comparisons were made between groups the control group exhibited a significantly greater preference for the high fat, moderate CHO (carbohydrate) product than either of the patient groups (controls: median, 7.0, IQR = 2.0 compared with BC: median, 5.0, IQR = 4.7,  $P < .01$ ; controls compared with HC: median, 6.0, IQR = 4.0,  $P < .01$ ). No differences in macronutrient preference between the BC and HC group was apparent.

**Analysis Within Groups.** Macronutrient preference was also examined within each group studied. The control group showed no significant preference for any macronutrient combination. The BC group showed a significant preference for the high fat, high CHO product (preferred high fat, high CHO to low fat, high CHO,  $P < .05$ ; preferred high fat, high CHO to high fat, moderate CHO,  $P < .01$ ). Hepatocellular patients showed a preference for low fat, moderate CHO when compared with low fat, high CHO ( $P < 0.05$ ) and high fat, moderate CHO ( $P < .01$ ). Additionally, the HC group preferred high fat, high CHO to high fat, moderate CHO ( $P < .05$ ) (Fig. 2).

To examine the association between fuel oxidation and macronutrient preference the relationship between oxidation rates and hedonic ratings was examined. In the control group a significant association was observed between the low fat, low CHO test stimuli and post-absorptive rate of glucose oxidation ( $r = .58$ ,  $P < .05$ ). In other words, the higher the rate of glucose oxidation the greater the preference for the ice

TABLE 2. Demographic, Clinical, and Nutritional Status of Control Subjects and Cirrhotic Patients

	Controls	All Cirrhotic Patients	Biliary Cirrhosis	Hepatocellular Cirrhosis
n	18	65	34	31
Male:Female	9:9	25:40	3:31	22:9
Age (years)	50.2 (2.7)	54.6 (2.2)	57.7 (1.6)	51.2 (1.7)
Child's Pugh score	—	8.7 (0.4)	8.3 (0.4)	9.0 (0.4)
Weight (kg)	76.9 (2.9)	66.7 (2.2)	62.0 (2.3)*	71.9 (2.0)
Body mass index	26.0 (0.9)	24.1 (0.8)	23.5 (0.9)	24.7 (0.6)
Pre-illness weight loss	0	-7.0 (1.3)	-7.7 (1.4)	-6.3 (2.0)
Triceps skinfold (%)	109 (9.2)	81 (4.2)	83 (6.2)	79 (5.6)†
Arm muscle circumference (%)	102 (3.3)	94 (1.6)	94 (2.5)	93 (1.8)

NOTE. Mean ( $\pm$ SEM).

\* $P < .001$  versus controls.

† $P < .05$  versus controls.



TABLE 3. Cirrhotic Patients Stratified by Etiology and Nutritional Status

	Cirrhotic Patients	Biliary Cirrhosis	Hepatocellular Cirrhosis	Normally Nourished	Under-nourished
s A	15	8	7	10	5
s B	26	15	11	13	12
s C	24	11	13	11	14
albumin (g/L) (reference >35 g/L)	30.8 (0.3)	32.2 (0.9)	30.0 (0.9)	30.3 (1.0)	30.8 (0.9)
bilirubin (μmol/L) (reference 2-17 μmol/L)	101.2 (11.1)	148.3 (17.7)*	54.2 (5.8)	108.3 (14.7)	92.9 (17.0)
ombin ratio†	1.3 (0.04)	1.2 (0.05)†	1.2 (0.05)	1.3 (0.05)	1.3 (0.05)
active protein (mg/L) (reference <10 mg/L)	10.2 (2.1)	11.4 (2.7)	8.7 (3.4)	11.7 (3.5)	8.5 (2.3)

TE. Mean (±SEM).

< .0001 versus hepatocellular.

< .01 versus hepatocellular.

patient value/control value.

n with the lowest energy content (low fat moderate ). In the BC group, a significant relationship was ve between the rate of lipid oxidation and preference low fat, high CHO test stimuli ( $r = .36$ ,  $P < .05$ ), fore, as the rate of lipid oxidation increases the prefer<sup>2</sup> for Low fat, high CHO increases. In HC patients no onship between lipid or carbohydrate oxidation rates macronutrient preference were observed. In addition, no onship between substrate oxidation rates and dietary e was seen in any study group. Similarly, no association een dietary intake and macronutrient preference was rved in control subjects or any patient group.

## DISCUSSION

e evidence that undernutrition is prevalent in chronic disease is unequivocal.<sup>32</sup> In this study, fat and lean tissue ves were determined by arm anthropometry, a technique remains sensitive in cirrhotic patients in whom there be a degree of fluid shift.<sup>33</sup> Preservation of muscle mass be explained by the fact that no patient had an acute plication of his or her disease or were recovering from an tive episode. The lower protein oxidation rates observed e clinically stable cirrhotic patients along with anthropo- c results suggest endogenous protein sparing. Other rchers have reported depleted muscle mass in their otic patients. However, these have invariably been per- ed in patients recovering from a complication of liver se<sup>34-35</sup> and loss of muscle mass is not a consistent finding ng cirrhotic patients.<sup>4</sup>

ergy and macronutrient intakes were significantly lower e cirrhotic patient group when compared with controls. nteresting finding was the differences in dietary profiles

observed in patients with different causes of cirrhosis. Accept- ing that there are clinical similarities between patients with predominantly hepatocellular and cholestatic liver disease, the present study does suggest there are important metabolic differences between the studied groups. This is supported by a recent study which examined the influence of etiology on the response to feeding.<sup>36</sup>

Compared with control subjects energy intakes were lower in BC ( $P < .05$ ) and HC patients ( $P < .01$ ). Whereas daily energy intake was depressed in both patient groups, only HC revealed all macronutrients and total energy to be different from controls. In BC patients only protein and energy were different to controls. Consumption of the major metabolic fuels (fat and CHO) was not significantly different. Analysis of macronutrient intake between patient groups was not significantly different but all intakes of dietary components were lowest in HC patients. If extrapolated over a 9-month period, this would result in an energy deficit of 30 megajoules or a loss of approximately 1 kg of adipose tissue.<sup>37</sup> Clearly this is an oversimplification of long term energy dynamics in these patients with chronic disease but it may in part explain the greater loss of fat mass found in HC patients. The inability of c: rhotic patients to achieve an adequate energy intake was also implied from between-group food preference studies with control subjects exhibiting a preference for the high fat, moderate carbohydrate test stimuli than either patient group.

In the present study, the reduced CHO intake found, particularly in HC patients, suggests that emphasis on a high CHO dietary regimen may be unacceptable to these patients. Disturbances in carbohydrate metabolism and the metabolic sequelae in response to feeding have been widely reported in liver cirrhosis.<sup>36,38-40</sup> However, increased emphasis on exog-

TABLE 4. Energy and Macronutrient Intake in Controls, Biliary Cirrhosis, and Hepatocellular Cirrhosis

	ESPEN Guidelines (based on 67-kg pt)	Controls	All Cirrhotic Patients	Biliary Cirrhosis	Hepatocellular Cirrhosis
		16	52	26	26
n (g)	85.0	81.3 ± 2.9	58.3 ± 2.9†	61.4 ± 3.9*	55.6 ± 4.1†
	—	83.7 ± 6.2	63.2 ± 3.2†	65.3 ± 4.8	61.3 ± 4.4*
hydrate (g)	—	259 ± 11.4	203 ± 10.0†	211 ± 15.6	195 ± 13.3*
ol (g)	—	19.7 ± 5.7	0	0	0
er (MJ)	11.1	9.0 ± 0.5	6.8 ± 0.3†	7.0 ± 0.4*	6.5 ± 0.4†

n (±SEM).

< .05 versus controls.

< .01 versus controls.

< .001 versus controls.

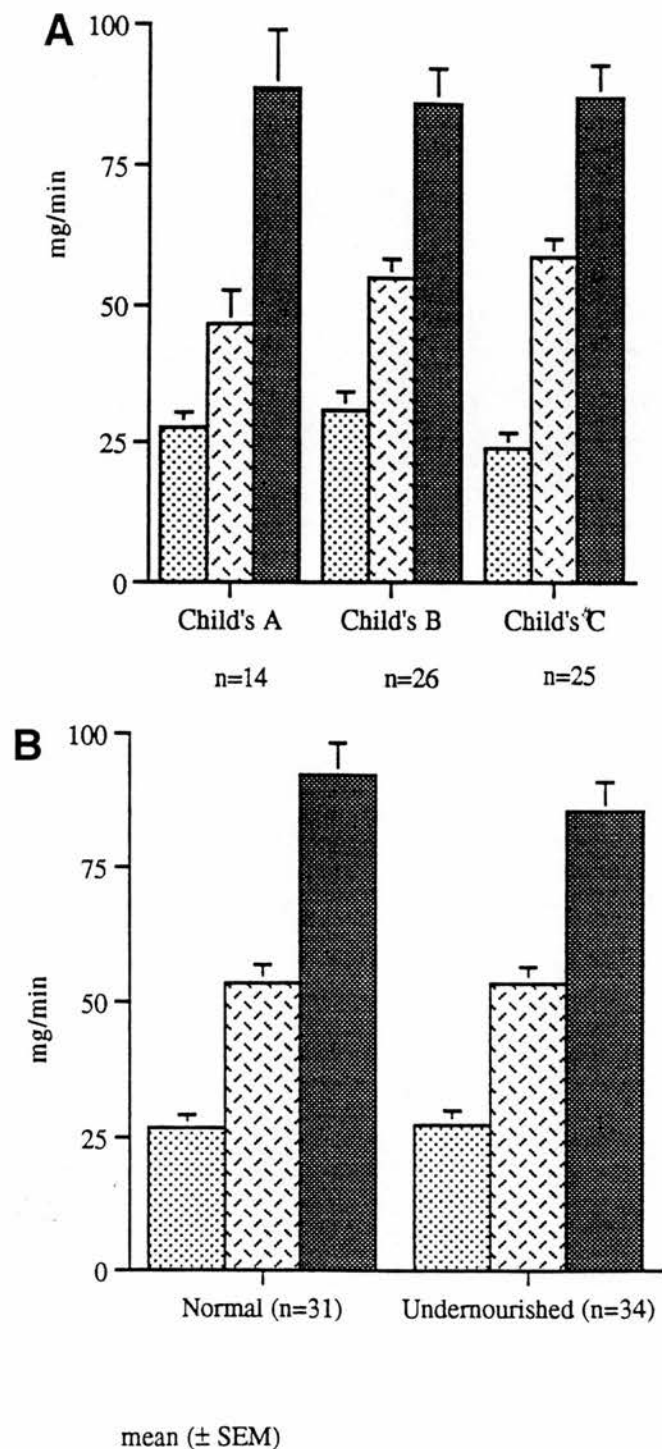


FIG. 1. Fasting substrate oxidation rates in cirrhotic patients classified by Child's Pugh Score and nutritional status.  $\square$ , protein (mg/min);  $\square$ , lipid (mg/min);  $\blacksquare$ , glucose (mg/min).

enous fat intake may facilitate patient compliance and consequently increase energy intake, there does remain some concern as to the ability of cirrhotic patients to handle this macronutrient. Previous research has suggested that defects in fat metabolism found in cirrhotic patients are a result of impaired clearance of triglycerides, carnitine deficiency, or reduced bile flow.<sup>41-43</sup> There is, however, no substantive clinical evidence to support the restriction of exogenous fat in

these patients,<sup>43</sup> particularly when it has been shown that lipid clearance rates are normal in cirrhotics.<sup>44</sup>

The depressed rate of glucose oxidation observed in fasting BC patients may reflect reduced hepatic glycogen capacity but appears not to be as exaggerated as the fasting profile observed in patients with HC cirrhosis, although this cannot be confirmed. The significant increase in postabsorptive rates of lipid oxidation found in HC patients is similar to that found by other investigators<sup>1,45</sup> and may be attributed to exhausted rather than depleted glycogen reserves. It is likely that this increased rate of lipid oxidation is an adaptive process, acting to offset the reduction in energy normally available through glycogenolysis. This adaptive mechanism will serve to preserve lean body mass at the expense of endogenous fat stores.<sup>4</sup> Increased lipolysis in the presence of an energy deficit will deplete body fat reserves. This may explain the lower endogenous fat reserves found in HC patients, exhibiting greater rates of lipolysis. No significant depression of muscle mass was observed in this group of stable cirrhotic patients in whom there was no evidence of an inflammatory response.

In healthy subjects, changes in physiological state lead to alterations in food preference, as evidenced, for example, with nutrient deprivation and weight loss, where there is an increased preference for fat.<sup>46-49</sup> In the current study, between-group analysis revealed that controls exhibited a preference for the high fat, moderate CHO food compared with cirrhotic patients. This may simply reflect the highly palatable properties of fat to this healthy population.<sup>50</sup> However, macronutrient preference within patient groups showed the BC group to have a preference for the high fat, high CHO food. This finding was surprising, as the dietary intake of fat is often prescriptively reduced in this patient group. It may be postulated that the insidious malabsorption of cholestatic disease could lead to the development of an appetite for high fat foods. The results of the present study are in contrast to the findings of Deems and Friedman,<sup>42</sup> who reported a reduction in fat intake in bile duct-ligated rats when compared with a group of control animals. It should be emphasized that ligation of the bile duct will induce acute cholestasis and does not mimic the slow, progressive cholestatic picture found in BC patients. In the present study, the preference for the high fat, high CHO stimuli evident in BC patients may reflect a drive to consume sufficient energy to maintain endogenous energy reserves.<sup>49</sup> However, the energy intake of BC patients was significantly lower than control subjects and suggests that in liver disease sensory food preference is not reflected in quantitative intake.

The macronutrient preference and dietary intake was different in HC patients. A significant preference for the low fat, moderate CHO food product was observed and only a marginal preference for high fat, high CHO when compared with the high fat, moderate CHO product. The latter finding could be due to the palatability attributes of the high fat, high CHO test stimuli that may act by masking the CHO content of test stimuli<sup>29</sup> and may not directly be associated with quantitative intake. This is supported by the finding that dietary analysis showed that the CHO and fat intake of HC group was significantly lower than controls and implies that these patients may consume energy-dense foods but only in small quantities. This may reflect a physiological inability by HC patients to normally metabolize these energy providing

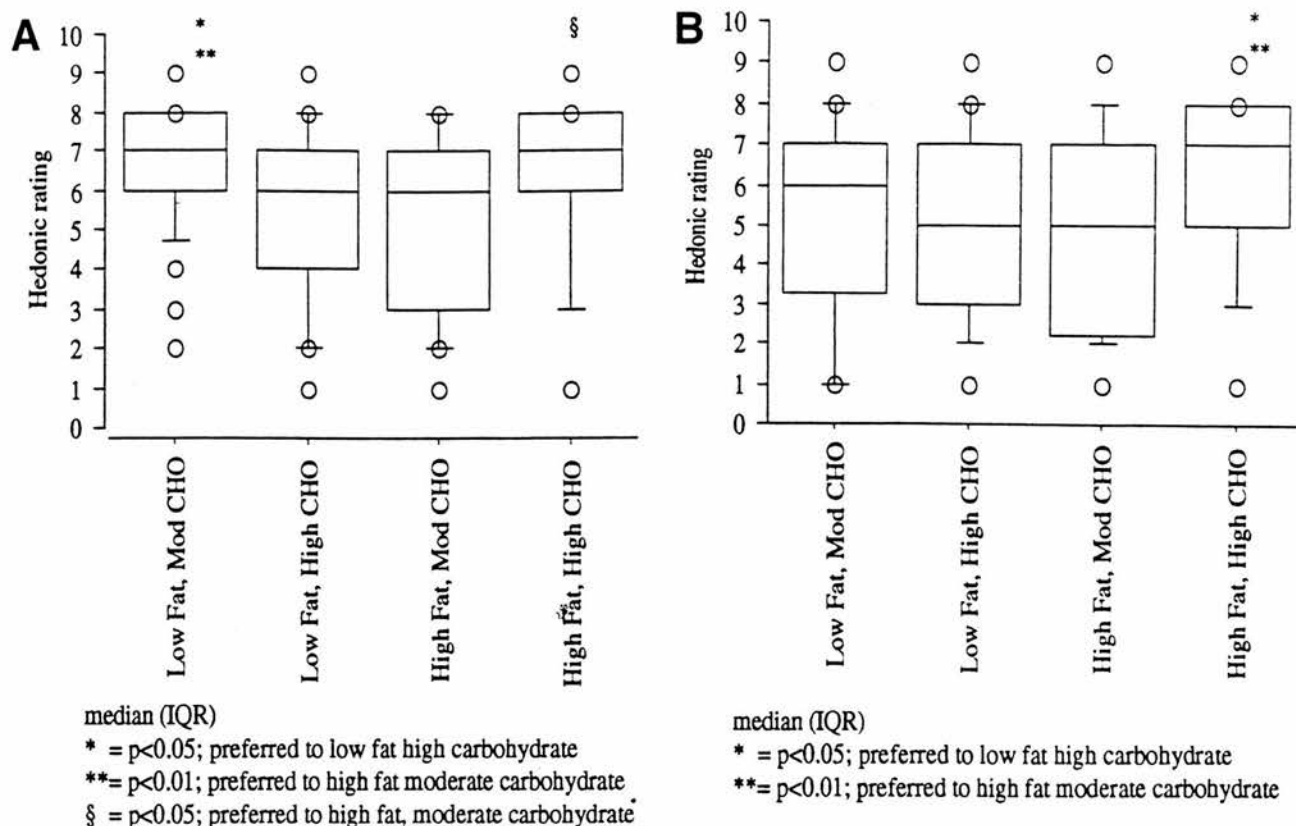


FIG. 2. Hedonic rating for macronutrient preference in biliary and hepatocellular cirrhotic patients. Data are represented as median and IQR with those ratings out with the 25th and 75th percentile represented individually: □, protein (mg/min); ▨, lipid (mg/min); ■, glucose (mg/min).

macronutrients and calls into question the effectiveness of the high-energy dietary regimen traditionally prescribed for patients with cirrhosis.

The relationship between substrate oxidation and macronutrient selection has been an area of considerable interest in obesity research.<sup>51</sup> The present study revealed that an association between fasting glucose oxidation rate and preference for the low fat, low CHO food in control subjects. This suggests that as the availability of glucose increases the preference for the low-energy food product increases but is not reflected in the dietary intake of these unrestrained eaters. In BC patients the association between lipid oxidation rate and preference for low fat, high CHO food suggests they may select these foods after periods of fasting when lipid oxidation rates increase. It is tempting to speculate that their improved ability to handle CHO<sup>52</sup> reflects better glycogen storage compared with HC patients and may be beneficial in preserving fat stores. This may also explain the fact that CHO intakes were not significantly lower in BC patients compared with controls, but the HC intakes were. Further studies are required to clarify these associations.

The significant relationship between rates of oxidative metabolism and food preference observed in the current study is impressive. After all, substrate oxidation is dictated by the body's requirement to regenerate adenosine triphosphate (ATP).<sup>51</sup> Fasted control subjects would continue to rely on hepatic glycogen reserves as an efficient ATP provider and may account for the association between CHO oxidation and low fat, moderate CHO food preference. It is not surprising that no relationship between fasting substrate

oxidation and dietary intake was seen in the present study as a relationship between these variables is only observed when oxidation rates are determined over a 24-hour period.<sup>53</sup> Further clinical studies are required to examine the relationship between substrate utilization and intake but the acceptability of the required methodology may prove an obstacle to patient recruitment. In addition, the melee of biological processes which accompany cirrhosis may serve to distort the relationship between oxidative metabolism and qualitative and quantitative components of ingestive behavior.

There has been considerable interest in the mechanisms involved in hepatic undernutrition and its impact on outcome. Although little attention has been paid to the causes of anorexia in chronic liver disease, it will, in the long term, detrimentally impact on nutritional status. There remains some debate as to exactly how the brain regulates food intake and indeed how the mechanisms involved in the integration of ingestive behavior and complementary biological processes act to maintain energy balance.<sup>54</sup> The present study has for the first time adopted an integrative approach in examining the association between whole body fuel oxidation rates and ingestive behavior in cirrhotic patients. It remains unclear whether associated nutrient deficits are metabolically driven and dictated by primary etiology.

#### REFERENCES

1. Greco AV, Mingrone G, Benedetti G, Capristo E, Tataranni PA, Gasbarrini G. Daily energy and substrate metabolism in patients with cirrhosis. *HEPATOLOGY* 1998;27(2):346-350.



2. Crawford DH, Cuneo RC, Shepherd RW. Pathogenesis and assessment of malnutrition in liver disease. *J Gastroenterol and Hepatol* 1993;8:89-94.
3. Prijatmoko D, Strauss BJG, Lambert JR, Sievert W, Stroud DB, Wahlqvist ML, Katz B, et al. Early detection of protein depletion in alcoholic cirrhosis: role of body composition analysis. *Gastroenterology* 1993;5:1839-1845.
4. Merli M, Riggio O, Romiti A, Anosto F, Mango L, Pinto G, Savioli M, et al. Basal energy production rate and substrate use in stable cirrhotic patients. *HEPATOLOGY* 1990;12:106-112.
5. Campillo B, Phuong B, Porrun B, Devanlay M. Influence of liver failure, ascites and energy expenditure on the response to oral nutrition in alcoholic liver cirrhosis. *Nutrition* 1997;13:613-621.
6. Muller MJ, Lautz HU, Plogmann B, Burger M, Korber J, Schmidt F. Energy expenditure and substrate oxidation in patients with cirrhosis: the impact of cause, clinical staging and nutritional state. *HEPATOLOGY* 1992;15:783-793.
7. Guglielmi FW, Mastronuzzi T, De Marco M, Laddaga L, Panella C, Francavilla A. Oxidative metabolism in cirrhotic patients with and without hepatocellular carcinoma: effects of malnutrition. *HEPATOLOGY* 1992;16:1144-1149.
8. Crawford DHG, Shepherd RW, Halliday JW, Cooksley GW, Golding SD, Cheng WS, Powell LW. Body composition in non alcoholic cirrhosis: the effect of disease aetiology and severity on nutritional compartment. *Gastroenterology* 1994;106:1611-1617.
9. Merli M, Riggio O, Dally L, PINC. Does malnutrition affect survival in cirrhosis? *HEPATOLOGY* 1996;23:1041-1046.
10. Selberg O, Botcher J, Tusch G, Pichlmayr R, Henkel E, Muller M. Identification of high and low risk patients before liver transplantation: a prospective cohort study of nutritional and metabolic parameters in 150 patients. *HEPATOLOGY* 1997;25:652-657.
11. Nompelleggi DJ, Bonkovsky HL. Nutritional supplementation in chronic liver disease: an analytical review. *HEPATOLOGY* 1994;19:518-533.
12. Neilsen K, Kondrup J, Martinsen L, Stilling GB, Wikman B. Nutritional assessment and adequacy of dietary intake in hospitalised patients with alcoholic liver cirrhosis. *Brit J Nutr* 1993;69:665-679.
13. Cabre E, Gonzalez-Huix F, Abad-Lacruz A, Esteve M, Acero D, Fernandez-Baneres F, Xiol X, et al. Effect of enteral nutrition on the short term outcome of severely malnourished cirrhotics. *Gastroenterology* 1990;98:715-720.
14. Tordoff MG, Friedman MI. Hepatic portal glucose infusions decrease food intake and increase food preference. *Am J Physiol* 1986;192R:196R.
15. Friedman MI. Control of energy intake by energy metabolism. *Am J Clin Nutr* 1995;62:1096S-1100S.
16. Scharrer E, Del Prete E, Rossi R, Langhans W. Metabolic regulation of food intake. *Proc Soc Nutri Physiol* 1993;1:35-41.
17. Budilion G, Cuomo R, Taccone W, Panico G, Pumpo R, Jaquinto G, Manzillo G. Gastrointestinal peptide hormones in acute viral hepatitis. *Ita J Gastroenterol* 1996;28:86-90.
18. Mc Clain CJ, Hill D, Schmidt J, Diehl AM. Cytokines and alcoholic liver disease. *Sem Liver Dis* 1993;13:170-172.
19. Bergasa NV. Hypothesis: taste disorders in patients with liver disease may be mediated in the brain: potential mechanisms for a central phenomenon. *Am J Gastroenterol* 1998;93:1209-1210.
20. Deems RO, Friedman MI, Friedman LS, Munoz SJ, Maddrey WC. Chemosensory function, food preference and appetite in human liver disease. *Appetite* 1993;20:209-216.
21. Madden A, Bradbury W, Morgan M. Taste perception in cirrhosis: its relationship to circulating macronutrients and food preferences. *HEPATOLOGY* 1997;26:40-48.
22. Drewnowski A, Shranger E, Lipsky C, Stellar E, Greenwood M. Sugar and fat: sensory and hedonic evaluation of liquid and solid foods. *Physiol Behav* 1989;45:177-183.
23. Smith FR, Henkin RJ, Dell RB. Disordered gustatory acuity in liver disease. *Gastroenterology* 1976;70:568-571.
24. Pugh RNH, Murray-Lyon IM, Dawson JL, Pretroni MC, Williams R. Transection of the oesophagus for bleeding oesophageal varices. *Br J Surg* 1973;60:646-649.
25. Nettleton DA, Day KC, Nelson N. Dietary survey methods. A comparison of nutrient intakes within families assessed by household measures and the sern weighed method. *Journal Human Nutrition* 1980;34:349-354.
26. Jelliffe DB. The assessment of the nutritional status of the community. *WHO Monograph* 1996:53.
27. Bristrian BR, Blackburn GL, Vitale J, Cochran D, Naylor J. Prevalence of malnutrition in general medical patients. *JAMA* 1976;235:1567-1570.
28. Acheson KJ, Schutz Y, Bessard T, Ravussin E, Jequier E. Nutritional influences on lipogenesis and thermogenesis after a carbohydrate meal. *Am J Physiol* 1984;246:62E-70E.
29. Drewnowski A, Greenwood MRC. Cream and Sugar: human preferences for high fat foods. *Physiol Behav* 1983;30:629-633.
30. Peryam DR, Pilgrim FJ. Hedonic scale method of measuring food preference. *Food Technology* 1957;11:9-14.
31. Plauth M, Merli M, Kondrup J, Weimann A, Ferenci P, Muller MJ. ESPEN guidelines for nutrition in liver disease and transplantation. *Clin Nutr* 1997;16:43-55.
32. Kondrup J, Muller MJ. Energy and protein requirements of patients with chronic liver disease. *HEPATOLOGY* 1997;27:239-247.
33. Loguercio C, Sava E, Marmo R, Blanco CV, Coltori M. Malnutrition in cirrhotic patients: anthropometric measurements as a method of assessing nutritional status. *Brit J Clin Prac* 1989;44:89-101.
34. Campillo B, Borres PN, Leluan M, Pornin B, Devanley M, Fouet P. Short-term changes in energy metabolism after 1 month of a regular oral diet in severely malnourished cirrhotic patients. *Metabolism* 1995;44:765.
35. Selberg O, Radoch E, Walter GF, Miller MJ. Skeletal muscle glycogen content in patients with cirrhosis. *HEPATOLOGY* 1994;20:135-141.
36. Mela DJ, Rogers PJ. Control of appetite and energy balance and imbalance. In: Mela DJ, Rogers PJ, eds. *Food Energy and Obesity*. London: Chapman Hall 1998:57-59.
37. Nolte W, Hartmann H, Ramadori G. Glucose metabolism and liver cirrhosis. *Exp Clin Endocrinol* 1995;103:63-74.
38. Petrides AS, Vogt C, Schulze-Berge D, Matthews DE, Strohmeyer G. Pathogenesis of glucose intolerance and diabetes mellitus in cirrhosis. *HEPATOLOGY* 1994;19:616-627.
39. Nosadine R, Avogavo A, Mollo F, Marescotti C, Tiergio A, Duner E, Merkel C, et al. Carbohydrate and lipid metabolism in cirrhosis: evidence that hepatic uptake of gluconeogenic precursors and of free fatty acids depends on effective hepatic flow. *J Clin Endocrinol Metab* 1984;58:1125-1132.
40. Richardson R, Davidson H, Hinds A, Rae P, Garden OJ. The influence of the metabolic sequelae of liver cirrhosis on nutritional intake. *Am J Clin Nutr* 1999;69:331-337.
41. Seidel D. Lipoproteins and liver disease. *J Clin Chem Clin Biochem* 1987;25:514-555.
42. Deems RO, Friedman MI. Macronutrient selection in an animal model of cholestatic liver disease. *Appetite* 1988;11:73-80.
43. Muller MJ, Reiger A, Willmann O, Lautz HU, Canzler H, Bolks HJ. Metabolic responses to lipid infusions in patients with liver cirrhosis. *Clin Nutr* 1992;11:193-206.
44. Druml W, Fischer M, Pidlich J, Lenz K. Fat elimination in chronic hepatic failure: long chain versus medium chain triglycerides. *Am J Clin Nutr* 1995;61:812-817.
45. Owen OE, Reichle FA, Mozzoli MA, Kreulen T, Patel MS, Elfenbein IB, Golsorkhi M, et al. Hepatic, gut and renal substrates flux rates in patients with hepatic cirrhosis. *J Clin Invest* 1981;68:240-252.
46. Tempel DL, Shor-Posner G, Dwyer D, Leibowitz SE. Nocturnal patterns of macronutrient intake in freely feeding and food deprived rats. *Am J Physiol* 1989;256:R541-R548.
47. Reed DR, Contreras RC, Maggio C, Greenwood MRC, Rodin J. Weight cycling in female rats increases dietary fat selection and adiposity. *Physiol Behav* 1988;42:389-395.
48. Drewnowski A, Holden WJ. Taste responses and food preferences in obese women: effects of weight cycling. *Int J Obesity* 1992;16(9):639-648.
49. Mela DJ, Rogers PJ. Control of appetite and energy balance and imbalance. In: Mela DJ, Rogers PJ, eds. *Food, Eating and Obesity*. London: Chapman and Hill, 1998:1-13.
50. Mela DJ. Understanding fat preferences and consumption: applications of behavioural sciences to a nutritional problem. *Proc Nutr Soc* 54:453-464.
51. Astrup A, Buemann B, Toubro S, Raben A. Defects in substrate oxidation involved in the predisposition to obesity. *Proc Nut Soc* 1996;55:817-828.
52. Taylor R, Heine RJ, Collins J, James OFW, Alberti KGMM. Insulin action in cirrhosis. *HEPATOLOGY* 1985;5:64-71.
53. Thomas CD, Peters JC, Reed GW, Abumrad NN, Sun M, Hill JO. Nutrient balance and energy expenditure during ad libitum feeding of high fat and high carbohydrate diets in humans. *Am J Clin Nutr* 1992;55:934-942.
54. Langhans W. Role of the liver in the metabolic control of eating: what we know and what we do not know. *Neurosci Behav Rev* 1996;20:145-153.

SEP 01 1999  
BY \_\_\_\_\_



1 September 1999

DEPARTMENT of CLINICAL and SURGICAL SCIENCES  
Surgery

Dr. R.A. Weisiger,  
Associate Editor,  
Hepatology,  
1360 9th Avenue,  
Suite 220,  
San Francisco,  
CA 94122,  
USA.

SEP 03 1999

The University of Edinburgh  
Royal Infirmary  
Laureston Place  
Edinburgh EH3 9YW  
Fax 0131 228 2661  
Email: OJGarden@ed.ac.uk  
Direct dial 0131 536 3812

Dear Dr. Weisiger,

I write requesting copyright permission for the inclusion of the paper cited below in my PhD submission.

Davidson HIM, Richardson R, Sutherland D, Garden OJ  
Macronutrient preference, dietary intake, and substrate oxidation among  
stable cirrhotic patients.  
Hepatology 29, 1380-1386, 1999.

I look forward to hearing from you.

Yours sincerely,

Rosemary A. Richardson,  
PhD Research Fellow to Professor Garden.



PERMISSION GRANTED for thesis only  
provided that:

you obtain the permission of the author(s);  
the material to be used has appeared in our  
publication without credit or  
acknowledgement to another source; proper  
credit is given

Date 9/20/99

By

Journal Permissions Department  
W.B. Saunders Company  
Orlando, Florida 32887

HEAD OF DEPARTMENT  
Professor of Hepatobiliary Surgery O James Garden